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PROGRESS

PHYTOTOXIC REACTIONS OF SOME BLOOD SERA*

WITH BIOMETRIC ANALYSES

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INTRODUCTION

NEARLY twenty years ago, while studying the general laws underlying physiologic and pharmacologic reactions, one of us (D. I. M.) conceived the idea of making comparative studies of the effect of various drugs and chemicals on living plant protoplasm in contrast to living animal test objects. By pharmacology, as it is taught in most of our medical and research institutions, is meant the investigation of the effects of drugs and poisons on living animals. Strictly speaking, it is *zoopharmacology*. However, just as the domain of physiology is subdivided into *zoo*physiology, i.e., study of physiologic functions of animals, and *phyto*physiology, or plant physiology, or study of physiologic functions of plants, so can the science of pharmacology be subdivided into *zoo*pharmacology and *plant* pharmacology, or study of the effects of drugs and poisons on living plants. Initial studies of this type, carried out by one of us (D. I. M.), led to such interesting findings that as a result there was developed a new department of science, to which was given the name of "*phyto*pharmacology."[1] A number of the discoveries made in the evolution of this branch of pharmacology have a direct bearing on clinical medicine and in the present paper we shall describe and explain some of these developments.

The effects of drugs and poisons on plant tissues may be studied in various ways. Thus the plant physiologist entering this field may study the effects of chemicals on germination of seeds, on growth of roots and stems, on flowering

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and flowers; the effects of various chemicals on geotropic and heliotropic properties of plants, on processes of respiration and transpiration, on growth of yeasts and fungi, on behavior of chloroblasts, on protoplasmic streaming, photosynthesis, oxidation and reduction phenomena, etc. For practical pharmacologic purposes it was found that quantitative phytopharmacologic studies could be most conveniently made by measuring the growth of roots and sometimes of stems of certain seedlings reared in artificial plant physiologic nutrient media. Growth of such seedlings in different plant physiologic solutions containing a definite proportion of the salts necessary for normal growth and of other seedlings of the same type reared in similar solutions to which small amounts of unknown drugs had been added, could then be quantitatively compared. For such studies various plant physiologists have found the seeds of the *Lupinus albus* very useful, and we and our collaborators have employed them extensively in our phytopharmacologic researches.

STUDIES ON COCAINE

The earliest phytopharmacologic studies, carried out by Macht and Livingston,[2, 3] dealt with the pharmacodynamic effects of the alkaloid cocaine and its products of hydrolysis on growth of *Lupinus albus* seedlings under standard ecologic conditions. It is well known that the cocaine molecule can be easily split by hydrolysis into three components, one a complicated nitrogenous base known as ecgonin, another, a molecule of a simple aromatic benzoic acid, and the third, a molecule of methyl alcohol; and that cocaine itself, or in the form of its simple salts, is a powerful poison for animals and animal tissues. Ecgonin is not as toxic for animals as cocaine itself; small doses of methyl alcohol are certainly not toxic for most lower animals; and benzoic acid as such, or in the form of its sodium salt, is practically nonpoisonous. A study of cocaine and its products on living seedlings of *Lupinus albus* by methods described elsewhere and to be discussed at length in the present paper, revealed that cocaine itself is not extremely poisonous for plant test objects. On the other hand, sodium benzoate, which is practically nonpoisonous for animal tissues, was very toxic for living plant protoplasm. A 2 per cent concentration of cocaine hydrochloride in the plant physiologic solutions was required to inhibit growth of *Lupinus albus* seedlings completely, while a 0.007 per cent solution of sodium benzoate accomplished the same result. It thus became apparent that while cocaine was much more poisonous for living animal tissue than for plant protoplasm, one of its components, sodium benzoate, was far more toxic for plant tissue than for living animals. This curious observation suggested that the presence of some toxic substances, in quantities not pronounced enough to affect animals, might be detected by means of living plants. Accordingly, one of us (D. I. M.) debated the feasibility of employing plants to determine the toxicity of certain bloods which could not be demonstrated by any other biological or physicochemical method. Such inquiry has been attended by more success than was ever anticipated.

STUDIES ON MENOTOXIN

The first phytopharmacologic studies undertaken on blood toxins were suggested by certain historical considerations. The belief is popular among all

races, ancient, medieval, and modern, that a woman at time of her menstruation is "unclean," using the word in a transitive sense as contaminating. Such a person was supposed to defile everything with which she came in contact. Thus, for instance, flowers touched by a menstruating woman withered; fruit that she canned became spoiled; dough that she kneaded failed to rise; and wine that she expressed from grapes soured. No careful investigation was made of the subject until 1920, when Professor Shick observed that beautiful, freshly cut flowers handled by a menstruating woman assistant commenced to fade soon after she had touched them. Reporting this circumstance and the similar results he had obtained in experiments with flowers in the hands of other menstruating women, Dr. Shick expressed the hope that some one would undertake to study the subject in a thoroughly scientific quantitative manner. Such a scientific investigation was carried on by Macht and Lubin,[4] who demonstrated quantitatively that small amounts of menstrual blood sera, as compared with normal sera of the same individuals, inhibited the growth of *Lupinus albus* seedlings. Moreover, the presence of this toxic substance, named "menotoxin" (by D. I. M.), was demonstrated by similar phytopharmacologic methods in the saliva, milk, urine, tears, and particularly the sweat, of menstruating women. These studies, published seventeen years ago, were later supplemented by those of Macht and Davis,[5] who showed that chemically menotoxin is probably akin to oxycholesterin. Their findings, which other investigators confirmed by different means, are now of special interest in connection with phytopharmacologic studies on the nature of the sex hormones, also found to be closely related in chemical structure to cholesterol. Most important of all, however, the first studies on menotoxin, instigated by the interest in medical history (D. I. M.), led to discovery of toxic reactions in the blood of patients suffering from various pathologic conditions. In the present paper we purpose to recapitulate the results already obtained in studies made on various types of blood sera and to describe the research we are now conducting and the phytotoxic reactions we discovered in bloods from other diseases.

TOXIC SERA

The phytopharmacologic method will be described in detail in the second part of this paper. Briefly, it consists of studying the root growth of *Lupinus albus* seedlings in normal plant physiologic solutions and comparing it with that of similar seedlings in the same kind of solution, to which had been added definite concentrations of the blood sera or plasma to be examined. The average root growth of *Lupinus albus* seedlings in twenty-four hours under standard conditions of temperature and light in a solution containing an unknown serum or chemical divided by the average increment of control roots is known as the *phytotoxic index* or *index of growth,* which may be expressed by the formula,

$$\text{Index} = \frac{X}{N}.$$

The average phytotoxic index of root growth of *Lupinus albus* seedlings in plant physiologic solutions containing small amounts of blood sera from hundreds of

normal individuals was found to be 70 to 75 per cent. Blood sera obtained from a number of women at onset of catamenia yielded an average phytotoxic index of 51 per cent. One of the most remarkable findings made in early phytopharmacologic studies was that obtained during investigation of sera from persons with various anemias. Blood sera of patients with pernicious anemia, male and female, definitely inhibited growth of *Lupinus albus* seedlings.[6, 7] Specimens from the first 50 patients examined yielded an average index of 44 per cent, a reading considerably lower than that given by menstrual blood. Such a phytotoxic effect was exerted not only by the blood but also by the spinal fluid[8] and stomach washings[9] from persons with pernicious anemia. Sera of certain other diseases, listed in Table I, also exhibited a characteristic phytotoxicity.

TABLE I

TOXIC SERA

KIND OF BLOOD SERUM	AVERAGE NUMBER OF CASES	PHYTOTOXIC INDEX (%)
Normal human	500	75
Menstrual	50	51
Pernicious anemia	50	44
Aplastic anemia	10	56
Pemphigus	200	59
Leprosy	22	47
Trachoma	100	48
Hodgkin's disease	10	60

TABLE II

OTHER ANEMIAS

KIND OF BLOOD SERUM	PHYTOTOXIC INDEX (%)
Severe secondary anemia	65 to 69
Carcinoma	70
Pellagra	70
Hemolytic jaundice	74
Obstructive jaundice	71
Malaria	70
Mononucleosis	80
Banti's disease	75
Lymphatic leucemia	68
Myelogenous leucemia	78
Sprue	60

Sera from patients with aplastic anemia gave an index of 50 per cent; those obtained from persons with leprosy, a reading of 47 per cent; from trachoma, 48 per cent; from Hodgkin's disease, 60 per cent. Most important of all, blood sera from the baffling skin disease, pemphigus, yielded an average phytotoxic index of 59 per cent. These phytotoxic reactions assume a profound significance when considered with the findings obtained in studies of sera from other forms of anemia and dermatoses.[10, 11] Table II exhibits the findings obtained with sera from secondary anemias, from the anemias of malignant disease, from various types of leucemia and certain other pathologic conditions. Thus was

established the availability of a test for differentiating between pernicious anemia and other types of anemia, between pemphigus and other forms of skin disease simulating it, and between trachoma, regarded by many as a systemic disease, and other varieties of conjunctivitis and localized eye disease.[12]

TABLE III

NONTOXIC SERA

KIND OF BLOOD SERUM	PHYTOTOXIC INDEX (%)
Syphilis	81
Tuberculosis	78
Scarlet fever	79
Measles	80
German measles	80
Varicella	80
Eclampsia	75
Postpuerperal (12 weeks)	80

Of equal interest were the *negative* findings derived from phytopharmacologic study of sera from certain grave pathologic conditions, i.e., syphilis, tuberculosis, scarlet fever, and other exanthemas, and also eclampsia. The phytotoxic indices of some of these nontoxic sera are shown in Table III. Tables I to III are obviously of clinical interest and throw some light on the etiology of certain diseases, such as pemphigus, pernicious anemia, trachoma and eclampsia. The phytopharmacologic test is also helpful in differentiating some pathologic conditions having certain points of resemblance. Thus, for instance, one of us by this method has differentiated between syphilis, tuberculosis, and leprosy. Further data on the subject may be found elsewhere. In this connection it is interesting to note that the average phytotoxic index of normal human blood specimens, 70 to 75 per cent, is practically the same as that obtained with sera of other mammalia, small and large, and of most fish and other cold-blooded animals with the exception of reptiles.

TABLE IV

SERA FROM VIRUS DISEASES

KIND OF BLOOD SERUM	ANIMAL INOCULATED	PHYTOTOXIC INDEX OF CONTROL (%)	PHYTOTOXIC INDEX OF VIRUS SERUM (%)
Fowl pox	Chicken	71	80
Vaccinia	Rabbit	73	80
Vaccinia	Rat	67	78
Herpes simplex	Rabbit	72	84
Virus fixé	Rabbit	75	61
Virus fixé	Guinea pig	81	65
Virus III	Rabbit	72	84
X-virus	Cat	70	65
Rous' tumor	Chicken	66	92
Shope's fibroma	Rabbit	77	96
Infectious myxomatosis	Rabbit	75	85
Rivers' lymphocytic choriomeningitis	Guinea pig	79	102
Poliomyelitis	Monkey	61	62
Tetanus toxin alone, 86 per cent	Rabbit	75	63
Strychnine nitrate, 93 per cent	Rabbit	77	61

ATOXIC SERA

The negative results obtained with sera of certain diseases listed in Table III suggested an investigation which was conducted by Macht and Gardner,[13] who studied blood specimens from various animals experimentally inoculated with a number of virus diseases.

Blood was taken from such animals before experiments were begun and later after the virus diseases were fully developed. It was found that sera from animals inoculated with the virus diseases yielded phytotoxic indices much higher than normal blood specimens from the same animals. Bloods from animals inoculated with virus fixé or virus hydrophobia were the only exception, and the increased toxicity of such specimens was shown to be due to convulsions produced by the virus because similar findings were made after injection of small amounts of strychnine and tetanus toxin (see Table IV).

BLOOD SERA FROM PEMPHIGUS, X-RAYED, AND PSYCHOTIC PATIENTS

During the past five years the phytopharmacologic test has been a routine procedure in various researches made in these laboratories. Such investigation has been made on the comparative toxicity of various synthetic inorganic and organic compounds, the growth hormones, the influence of vitamins on plant growth, the pharmacologic effects of sex hormones on growth of plants, and a search for toxins in various diseases of the blood. Special studies, described here in detail, were made on (1) blood sera from animals and human beings after their exposure to x-rays, (2) the toxicity of pemphigus blood sera, and (3) blood sera from patients with various organic and functional psychoses.

The first problem undertaken concerned the effects of prolonged administration of x-rays and had a direct bearing on the so-called roentgen sickness. Repeated experimentation revealed that from twenty-four to forty-eight hours after exposure of various animals to therapeutic doses of x-rays, a toxic substance was developed in their blood which could be demonstrated phytopharmacologically.[14] As compared with normal specimens that had been drawn from the same animals before treatment with x-rays, blood sera obtained afterward were quite toxic, but the toxicity varied with the region of the body irradiated. When these tests on normal animals had been completed, a similar series was begun with human beings, from whom blood sera were obtained both before and after their treatment with x-rays for malignant and nonmalignant pathologic conditions. It was found that specimens from patients who had been subjected to such exposure definitely inhibited growth of *Lupinus albus* seedlings. Further investigation revealed the fact that not only sera from patients treated with x-rays but also those from apparently healthy physicians, technicians, and attendants in roentgenologic clinics were definitely toxic for *Lupinus albus,* as compared with control specimens from unexposed normal individuals. A number of these findings have been published, but the subject itself, which is of great clinical importance, is still under investigation. Table V shows the striking data already obtained.

TABLE V

EFFECT OF X-RAYS ON BLOOD OF CLINICAL PATIENTS

CASE NO.	DIAGNOSIS	PHYTOTOXIC INDEX		TIME ELAPSING BETWEEN X-RAY TREATMENT AND TAKING OF SPECIMEN	DOSAGE EMPLOYED (r.)
		BEFORE TREATMENT (%)	AFTER TREATMENT (%)		
N 17	Carcinoma of breast	73	45		200
N 17	Carcinoma of ovary	74			No x-ray
M. B. M.	Hypertrophied tonsils	72	37	24 hours	333
		72	72	8 days	
105391	Carcinoma of breast	68	42	48 hours	265
180902	Carcinoma of cervix	52	45	48 hours	156
181248	Carcinoma of breast		47	48 hours	160
174802	Carcinoma of prostate	79	46	24 hours	260
175925	Syringomyelia	73	56	24 hours	101
159409	Myoma	61	41	24 hours	270
174976	Carcinoma of submaxillary gland	66	58	24 hours	198
169275	Eustachian salpingitis	69	30	24 hours	129
176084	Hypertrophic nasopharyngitis	72	41	24 hours	160
183874	Carcinoma of breast	84	40	24 hours	202
170813	Carcinoma of breast	66	58	48 hours	194
181423	Chancre of tongue	69	64	24 hours	2 × 160
Mrs. Y.	Carcinoma of breast	68	32	24 hours	300

We still conduct another phytopharmacologic study of pathologic blood sera, initiated a year ago by Dr. Max Hayman, then a resident physician at Springfield State Hospital, Sykesville, Md., and one of us (D. I. M.). This research consists of a systematic examination of blood sera from various types of functional and organic psychoses. A complete presentation of the findings will be published in due time after the blood sera from a larger number of cases have been studied. Such an investigation is in progress. Table VI shows the results obtained after repeated examination of sera from eight psychotic patients. The case of one such patient will be described in detail here.

TABLE VI

PHYTOTOXIC INDICES OF SOME PSYCHOTIC BLOOD SERA

PATIENT	DIAGNOSIS	PHYTOTOXIC INDEX (%)
E. S.	Schizophrenia, catatonic hypokinetic type	44
H. R.	Schizophrenia, catatonic hypokinetic type	42
F. N.	Schizophrenia, catatonic hyperkinetic type	58
D. H.	Schizophrenia, catatonic hyperkinetic type	58
C. H.	Involutional melancholia type	52
M. R.	Involutional melancholia type	50
B. B.	Manic-depressive type	54
J. H.	Manic-depressive type	57

By far the most extensive phytopharmacologic study, which is still in progress, has been that made by the dermatologist, Dr. Isaac R. Pels, and one of us (D. I. M.) on pemphigus blood sera, which exhibited more toxicity than specimens obtained from all other skin affections, with the exception of leprosy. In fact, the phytopharmacologic test has become not only a valuable aid in diagnosing and differentiating this strange malady from dermatitis

herpetiformis and other dermatoses, but also an important contribution to the étiology of pemphigus. It has been most useful in establishing a diagnosis in the early stages of pemphigus. Although pemphigus is a rare disease, Macht and Pels up to the present time have examined phytopharmacologically more than 2,600 specimens taken from various skin diseases and forwarded to them for differential diagnosis by physicians in the United States and Canada. Routine phytopharmacologic examination of these blood sera has resulted in a positive diagnosis of pemphigus in fully 90 per cent of the cases, a diagnosis which was confirmed by clinical findings as the disease progressed. This figure in itself is incontrovertible proof of the value and reliability of the phytopharmacologic test for this disease. Because of the growing importance and practical value of the phytotoxic reaction in routine examination of blood sera from various diseases, and because of the difficulties some clinicians experience in carrying out the test, we purpose to describe it in considerable detail and to evaluate its reliability in the light of the latest biological and biometric information available.

METHOD OF EXPERIMENTATION

The method of detecting toxic reactions in blood sera of pernicious anemia, pemphigus, and other pathologic conditions consists of determining quantitatively the increment in length of the single, well-defined, straight roots of seedlings of *Lupinus albus,* after their growth for twenty-four hours in a plant physiologic (Shive) solution containing a small amount of the blood serum to be tested. The average root length of ten or more such seedlings is measured, and the plants are immersed in a suitable plant physiologic nutrient solution containing all the salts or electrolytes necessary for perfect growth. Seedlings are grown in such solutions for twenty-four hours under standard conditions of light and temperature, after which the average increment in root length is determined. These seedlings grown in Shive solution are compared with similar seedlings placed for the same number of hours in the same kind of plant physiologic solution containing, in addition, a definite quantity of an unknown chemical, blood serum, or other substance to be tested. When a pathologic specimen of blood is examined, the growth of seedlings is determined not only for one group in plant physiologic solution alone, but also for another in Shive solution containing a small percentage of normal blood serum. It is customary in all phytopharmacologic work to employ 1 per cent solutions of blood sera.

To make a correct phytopharmacologic test a great deal of training is required not only in ordinary pharmacologic methods, but also in laboratory technique pertaining to plant physiology. Beginners who anticipate success with the first attempt at such experimentation will probably be disappointed. Without previous practice even experienced laboratory workers have occasionally been unable to germinate the seeds, much less to raise the seedlings and measure them in phytopharmacologic tests. In this highly specialized work, as in any other complicated laboratory technique, negative results in the hands of a tyro count for little or nothing.

In examining specimens for pemphigus toxin, fresh blood serum is almost invariably employed in making the test although, as Macht and Grumbein have pointed out,[15] dried specimens of whole pemphigus blood or of blood serum retain much of the toxin and may be used for phytopharmacologic examination when necessary. Occasionally, serum from bullae is also tested for the pemphigus toxin. A 1 per cent solution is prepared by dissolving the serum in equal parts of distilled water and Shive plant physiologic solution,[16] which consists of 10.4 c.c. of a 0.5 molar solution of chemically pure calcium nitrate, 30 c.c. of a 0.5 molar solution of chemically pure magnesium sulfate, and 36 c.c. of a 0.5 molar solution of diacid potassium phosphate (KH_2PO_4), to which is added enough distilled water to make one liter. In testing body fluids for clinical purposes, it is absolutely necessary to use salts of the highest chemical purity. Even the test tubes employed in such work should be of a special type, i.e., they should be made of hard or nonalkaline glass, in order that all conditions of experimentation may be rigidly controlled and standardized. The hydrogen-ion concentration of a mixture of equal parts of distilled water and Shive solution is usually about 5.4. When blood serum is added to make a 1 per cent solution, the pH shifts toward the alkaline side and varies from 5.4 to 5.6. Previous publications have shown that such slight variations in pH have little effect on the growth of seedlings immersed in the solutions and are certainly not sufficient to account for differences in toxicity between specimens of serum from both normal and diseased persons. As a matter of fact, there is usually no difference at all in hydrogen-ion concentration between a 1 per cent solution of serum from a normal individual and a similar solution from a diseased one.

In making the phytopharmacologic test, we employed a special apparatus containing twenty-three parallel rows of ten perforations for holding test tubes in an upright position. The ten tubes in the first row, and sometimes those in two or three such rows, are filled with control solutions consisting of equal parts of Shive saline solution and distilled water. Succeeding rows of ten tubes each are filled with 1 per cent solutions of various specimens of blood serum to be dissolved and tested in the plant physiologic saline solution. The *Lupinus albus* seedlings are then carefully selected, measured, and immersed in the solutions filling the upright tubes. For ordinary routine examinations an average of ten seedlings for each solution to be tested is usually sufficient for a single experiment. The test should be repeated on several consecutive days, however, until the phytotoxic indices differ from each other by not more than 5 per cent. Such repetition of the test, on two days at least, is necessary because occasional crops of *Lupinus albus* yield seedlings impervious to saline solutions of toxins. Such seedlings give a negative reading or an index considerably higher than that yielded by controls in normal blood serum. While it is true that specimens of serum obtained from patients with certain rare conditions (i.e., virus diseases, etc.) consistently give higher readings, such conclusions can be deduced only from several tests. In dealing with specimens of serum of patients suspected of having pemphigus, pernicious anemia, or some similar condition, negative or exceedingly high readings after a single examination should be regarded as "no takes" if in several repetitions of the test toxic effects are definitely exhibited.

The seeds employed for the phytopharmacologic test by the Macht-Livingston school of plant physiologists are those of *Lupinus albus,* large variety. The dry seeds, approximately 10 mm. in diameter, are obtained from Italy, where this variety of *Lupinus albus* is widely cultivated. The test can be made with the small varieties of *Lupinus albus,* approximately 6 mm. in diameter, which are grown in this country, but the percentage of germination and uniformity of size of these seeds are much less than those of the French or Italian varieties. Seeds of the giant *Lupinus albus,* 13 to 15 mm. in diameter, may also be employed in this work and have the advantage of possessing a very definite line of demarcation between stem and root. However, Macht and his associates have derived the best results from the large Italian variety of *Lupinus albus* seeds, which are remarkably hardy and will germinate even when five years old, although the most satisfactory results are obtained with seeds that are one to two years old. *Lupinus albus* seeds are soaked overnight in tap water. The next day the swollen seeds are ready for planting. Finely ground sphagnum was found to be the most suitable medium for the problem in hand. The sphagnum should not only be ground to a fine powder but should also be impregnated with just the amount of water most suitable for optimum germination. Here the beginner in phytopharmacology encounters his first difficulty. If the moss contains too much moisture, the seeds will rot, fungi will develop, germination will be defective, and the seedlings will be diseased. If it contains too little water, the seedlings will suffer from malnutrition and lack of turgor and will exhibit a stunted growth. A great deal of experimentation has proved that the best results are obtained with sphagnum containing from 70 to 80 per cent of water by weight. Such a medium can be successfully prepared only after considerable practice. Sphagnum prepared for the germination of seedlings must be rinsed several times with hot water to free it from an excess of acid matter, which would interfere with proper development of the plants. It must also be well aerated before it is deposited in the glass jars in which the seeds are to be planted. Swollen seeds, which have been soaked overnight, are planted closely together with the hili pointing down. In this way a large number of seedlings can be raised in a comparatively small area. A light layer of the moss is then sifted over the seeds, and the covered jar is allowed to stand in the dark for several days at room temperature. When the weather is mild, the seedlings are generally of convenient length for testing on the third day after planting. In winter, however, germination progresses more slowly even at room temperature, and the seedlings are not long enough until the fourth day after planting.

The most suitable seedlings for the phytopharmacologic test are those with roots measuring from 35 to 60 mm. Shorter seedlings may be employed, but their growth is not usually so rapid as that of the longer ones. For this reason, in recent years we have been discarding those less than 30 mm. long. On the other hand, much longer seedlings exhibit curvatures which make them difficult to measure. Healthy seedlings are taken out of the moss; the adhering particles are removed by gentle brushing or rinsing, and the length of their

roots is measured with a millimeter rule. Here the beginner encounters a second difficulty. While the line of demarcation between root and stem is usually definite and easy to find with the naked eye, with or without the aid of some innocuous staining solution, consistently accurate readings can be made only after much practice and experience. After the seedling has been measured, it is immersed in a tube of either the control solution or the blood serum solution to be examined. In this way ten seedlings are selected for each solution tested, including controls. The roots of the seedlings in any given experiment must be measured both at the beginning and at the end of the test by the same person. A woman experimenter should not handle the plants at the time of catamenia. All experimenters should wash their hands carefully before touching the objects to be tested.

An important detail of technique is the *matching* of seedlings, i.e., the selection of seedlings of as nearly the same length as possible. Experiments with living plants are generally more reliable than those with living animals because of the vastly larger number of the former available and the physical and economic obstacles to such wholesale experimentation with higher animals. To insure maximum accuracy in performing the phytopharmacologic test, however, the seedlings selected should be of as nearly the same length as possible. Especially should the individual plants in corresponding positions in different rows be closely matched. This is accomplished by filling the first tube of each row with seedlings of as nearly the same length as possible, and then filling the second tube in each row with similarly matched seedlings and matching in the same way all the plants in corresponding tubes in the remainder of the rows.

When such a procedure is followed, deviations in readings due to differences in growth between seedlings which are either too long or too short at the outset of the experiment are reduced to a minimum, and a more reliable comparison can be made with regard to the effect of various solutions on the growth of the roots.

After all the seedlings have been measured and allotted to their respective tubes, the outfit containing them is placed in a refrigerator or a low-temperature incubator. The seedlings are allowed to grow there in the dark for twenty-four hours, at the end of which time they are measured again, and the average increment of growth in control and unknown solutions is determined. The average growth of roots in any given solution of blood sera divided by the average increment in the length of roots of control seedlings grown in Shive solution alone and maintained under exactly the same light and temperature, gives the so-called phytotoxic index of growth for that serum. Thus blood from normal persons yields a phytotoxic index ranging from 70 to 75 per cent, and serum from patients with pemphigus, a reading of 58 or 59 per cent.

Perhaps the most important single factor affecting phytopharmacologic testing of serum from patients with pemphigus is the temperature. It is absolutely

necessary that seedlings grown in various solutions of serum be maintained at the temperature most suitable for the test. In earlier publications one of us (D. I. M.) specified 20° C. as the temperature most suitable for such work. Later experience, however, has shown that the best results in testing specimens of serum from patients with pemphigus are obtained with seedlings grown at a considerably lower temperature. The blood from such patients changes rapidly when kept at room temperature. The pemphigus toxin decomposes even at from 18° to 20° C. in the presence of the slightly acid Shive solution. Furthermore, the stimulus to growth of seedlings kept at temperatures higher than 15° C. or 60° F. is so great that slight inhibitions produced by the presence of small quantities of toxin are masked. On the other hand, when the seedlings are maintained at temperatures below 15° C., the growth of all, even those in normal control solutions, is greatly retarded; as a consequence, the phytotoxic indices yielded by specimens of both toxic and normal blood are much lower than they should be. In other words, seedlings grown in solutions at temperatures lower than 15° C. give more toxic readings than those grown in similar solutions at 15° C. Of all the ecologic factors playing roles in phytopharmacologic work with blood, temperature is the most important. Undoubtedly some investigators attempting the test have obtained discordant results because they did not regard this all-important ecologic factor. It is obvious, moreover, that all specimens of blood sent for phytopharmacologic testing, especially those from patients in whom pemphigus is suspected, should be protected from light, and, preferably, kept on ice. One of us (D. I. M.) has repeatedly observed that the red corpuscles of blood affected by pemphigus undergo decomposition much more rapidly than normal erythrocytes and effect a purple hemolyzed fluid quite unsuitable for phytopharmacologic examination.

For investigative purposes in connection with new problems involving determination of small differences in growth of seedlings placed in control and unknown solutions, respectively, the phytopharmacologic test can be made more accurate by "proving" the seeds and seedlings. In such cases, after seeds have been soaked and germinated in sphagnum, and the root growth of a large number has been measured, they are placed in solutions containing equal parts of distilled water and Shive. The growth of all these seedlings and their roots under standard conditions, i.e., in the dark and at a temperature of 60° F., is then determined, and only those are selected for further phytopharmacologic work which show approximately the same rate of growth, a procedure which eliminates plants weaker than others and renders the readings much more reliable. It is unnecessary to reiterate that the findings obtained by the writers with large numbers of proved seedlings are at least as reliable as those derived from any zoopharmacologic test. By use of the phytopharmacologic method it is possible to determine the toxicity of many unknown substances with various controls for hundreds of biological test objects grown simultaneously, a feat very difficult to accomplish in zoopharmacologic experimentation and possible then only at the cost of much more time, money, and labor in a highly subsidized laboratory.

STANDARD DEVIATION, PROBABLE ERRORS, AND CRITICAL RATIOS

Illustrative Experiments

To illustrate the reliability and utility of the phytopharmacologic test of various pathologic blood sera, five sets of experiments are detailed and analyzed by modern statistical methods.[17, 18] All five were carried on simultaneously under exactly the same conditions, and in each series there were 50 *Lupinus albus* seedlings with roots from 35 to 50 mm. in length. In the various solutions these seedlings were grown in the dark at 60° F. for twenty-four hours, at the end of which period the roots were measured again, and the phytotoxic indices were calculated. In Experiment A 50 control seedlings were grown in a mixture of equal parts of Shive and distilled water. Into the Shive solution, in which all the other series of seedlings were also grown, was introduced 1 per cent of (1) a normal human blood serum in Experiment B, of (2) a random specimen of blood from a patient tentatively diagnosed clinically as pemphigus in Experiment C, of (3) a serum obtained from a person after exposure to x-rays for nonmalignant disease in Experiment D, and of (4) a serum from a patient afflicted with schizophrenia of the hypokinetic type in Experiment E.

Experiment A

Increment in Root Length of 50 Controls in Shive Solution and Distilled Water (1:1)

17	16	14	12	13	13	13	18	12	14
13	15	13	15	16	9	13	14	13	14
15	12	15	9	11	13	11	11	13	13
12	10	15	12	13	15	12	14	16	11
14	9	10	11	13	13	13	11	12	15

Average increment in growth = 13.2 mm. σ (Standard deviation) $= \sqrt{\frac{\Sigma D^2}{N}} = 2.0.$

P.E. (Probable error) $= \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.19.$

Experiment B

Increment in Root Length of 50 Seedlings in Shive Solution with 1 Per Cent Normal Blood Serum

9	8	8	10	7	7	8	9	9	10
14	12	8	9	10	10	10	7	11	10
9	10	13	12	8	9	11	11	9	10
11	9	13	7	10	12	11	10	11	12
9	12	9	7	7	12	12	7	10	9

Average increment = 9.5 mm. Phytotoxic index of normal blood serum = 72 per cent.

$$\sigma = \sqrt{\frac{\Sigma D^2}{N}} = 1.8$$

$$\text{P. E.} = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.22$$

$$\text{P. E. diff.} = \sqrt{\overline{PE^2}_{\text{Shive}} + \overline{PE^2}_{\text{Normal serum}}} = 0.254$$

Correlation of Shive Solution and Normal Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{\text{P. E. diff.}} = 14.5$$

EXPERIMENT C

Increment in Root Length of 50 Seedlings in Shive Solution With 1 Per Cent of Pemphigus Serum

6	5	5	6	5	5	7	5	5	5
6	5	2	5	7	4	6	4	4	3
7	5	7	4	5	4	7	7	6	5
6	4	4	5	5	3	5	4	6	5
6	2	3	8	7	8	6	4	5	7

Average increment = 5.2 mm. Phytotoxic index of pemphigus serum = 40 per cent.

$$\sigma = \sqrt{\frac{\Sigma D^2}{N}} = 1.4$$

$$\text{P. E.} = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.134$$

$$\text{P. E. diff.} = \sqrt{\underset{\text{Normal serum}}{\overline{PE^2}} + \underset{\text{Pemphigus serum}}{\overline{PE^2}}} = 0.216$$

Correlation of Normal Serum and Pemphigus Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{\text{P. E. diff.}} = 19$$

EXPERIMENT D

Increment in Root Length of 50 Seedlings in 1 Per Cent Blood Serum, Obtained After X-ray Therapy

9	8	7	5	8	5	8	6	7	6
3	7	6	4	5	3	7	9	7	5
2	7	6	7	4	7	9	6	7	9
5	6	6	9	6	8	2	3	8	2
7	7	4	7	9	4	2	4	5	5

Average increment = 6.0 mm. Phytotoxic index of x-ray blood serum = 46 per cent.

$$\sigma = \sqrt{\frac{\Sigma D^2}{N}} = 0.2$$

$$\text{P. E.} = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.019$$

$$\text{P. E. diff.} = \sqrt{\underset{\text{Normal serum}}{\overline{PE^2}} + \underset{\text{X-ray serum}}{\overline{PE^2}}} = 0.17$$

Correlation of Normal Serum and X-ray Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{\text{P. E. diff.}} = 25$$

EXPERIMENT E

Increment in Root Length of 50 Seedlings in 1 Per Cent Blood Serum. Obtained From a Psychotic Patient

9	6	7	2	7	4	9	8	8	5
6	3	7	5	5	5	2	7	9	5
6	7	6	3	6	2	9	3	7	5
6	7	6	7	7	9	6	5	5	4
6	6	9	4	7	5	8	5	7	6

Average increment = 6.0 mm. Phytotoxic index of psychotic serum = 46 per cent.

$$\sigma = \sqrt{\frac{\Sigma D^2}{N}} = 0.183$$

$$\text{P. E.} = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.017$$

$$\text{P. E. diff.} = \sqrt{\underset{\text{Normal serum}}{\overline{PE^2}} + \underset{\text{Psychotic serum}}{\overline{PE^2}}} = 0.17$$

Correlation of Normal Serum and Psychotic Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{\text{P. E. diff.}} = 25$$

Since according to recognized mathematical criteria a critical ratio of 3.0 or more indicates that the difference between averages is a significant and not a chance difference, it will be seen that all the experiments analyzed to determine the standard deviation, probable error, and probable error of difference yield critical ratios of a much higher order and, therefore, reveal results of very high significance and reliability indeed.

ANALYSIS OF INTERGROUP VERSUS INTRAGROUP VARIATIONS BY FISHER'S METHOD

To further test the reliability of the foregoing data special analysis was made of the readings obtained for intergroup versus intragroup variations according to Fisher's well-known method.

Analysis of Variance.—Fundamentally analysis of variance is a test of significance. It is based on the solution of a number of problems of statistical distribution obtained by Fisher.[19] The total variation in this experiment is separated into two parts, viz. (1) the variation attributed to irregularities in the results obtained in the given group, and (2) the variation attributed to the difference between the mean performances of the different groups. Once these two variabilities have been separated, they are compared to see whether or not there is a significant difference between them. In this way it can be determined whether the variation within the group is significantly greater or less than the variation between groups.

1. *Variation Within the Group.*—(a) The sums of the squares of the deviations from the individual means are computed. In this case they are:

Shive solution	=	200.960
Normal blood sera	=	181.090
Pemphigus blood sera	=	96.000

(b) The sums of these squares pooled are equal to 478.050. (c) The variance from this source is estimated by dividing the pooled sums of the squares by the degrees of freedom. The latter is the product of the number of group and one less the number of trials, i.e., $3 \times 49 = 147$. Thus the estimate of variance is $478.050/147 = 3.252$.

2. *Variation Between Groups.*—(a) The sum of the squares of the deviations of the sums from the mean of the sums is computed. Since the latter is the sum of the sums over $3 = 472.33$, the step is as follows:

$$(496 - 472.33)^2 + (661 - 472.33)^2 + (260 - 472.33)^2 = 79.155$$

(b) This sum is divided by the number of trials per subject to reduce the sum of squares to a per item basis; thus $79155/50 = 1583.1$. (c) The variance is estimated from this source by dividing the reduced sum of squares by the degrees of freedom of groups, i.e., by $n - 1 = 2$; thus $1583.1/2 = 791.55$.

TABLE VII

COMPARISON OF READINGS OBTAINED WITH SEEDLINGS GROWN IN (1) SHIVE SOLUTION AND IN SOLUTIONS OF (2) NORMAL HUMAN BLOOD SERUM AND OF (3) PEMPHIGUS SERUM

SEEDLING	GROUP			TOTAL
	SHIVE	NORMAL BLOOD	PEMPHIGUS BLOOD	
1	17	9	6	
2	13	14	6	
3	15	9	7	
4	12	11	6	
5	14	9	6	
6	16	8	5	
7	15	12	5	
8	12	10	5	
9	10	9	4	
10	9	12	2	
11	14	8	5	
12	13	8	2	
13	15	13	7	
14	15	13	4	
15	10	9	3	
16	12	10	6	
17	15	9	5	
18	9	12	4	
19	12	7	5	
20	11	7	8	
21	13	7	5	
22	16	10	7	
23	11	8	5	
24	13	10	5	
25	13	7	7	
26	13	7	5	
27	9	10	4	
28	13	9	4	
29	15	12	3	
30	13	12	8	
31	13	8	7	
32	13	7	6	
33	11	11	7	
34	12	11	5	
35	13	12	6	
36	18	9	5	
37	14	7	4	
38	11	11	7	
39	14	10	4	
40	11	7	4	
41	12	9	5	
42	13	11	4	
43	13	9	6	
44	16	11	6	
45	12	10	5	
46	14	10	5	
47	14	10	3	
48	13	10	5	
49	11	12	5	
50	15	9	7	
Sums	661	496	260	1417
Means	13.22	9.92	5.20	28.34
Σ (deviations from means)^2	200.960	181.090	96.000	478.050

3. *Application of the F Test.*—This is that method described by Fisher[20] as "testing whether one estimate of variance derived from n_1 degrees of freedom is significantly greater than a second such estimate derived from n_2 degrees of freedom." A convenient adaptation is supplied by Mahalanobis[21] and Snedecor.[22] There are two steps in this test: the first is to form the ratio of the greater to the lesser variance, i.e., 791.55/3.252 = 243.5; the second is to match this value in Fisher's table for the distribution of F, a table which shows how high the ratio must be to be barely significant and highly significant. It is entered with the degrees of freedom of the two variances forming the ratio. In evaluating the significance of the two degrees of freedom in this experiment, as outlined above, the figure 3.06 is regarded as barely significant and the figure 4.75 as highly significant. Thus the ratio of 243.5, which is far in excess of the higher ratio of 4.75, shows that the difference between the groups in this experiment is very significant indeed and greater than that within the groups.

COMPARISON OF READINGS OBTAINED WITH SEEDLINGS GROWN (1) IN SHIVE SOLUTION ALONE, (2) IN SHIVE SOLUTION WITH 1 PER CENT NORMAL BLOOD SERUM, AND (3) IN SHIVE SOLUTION WITH 1 PER CENT X-RAY BLOOD SERUM

	SHIVE SOLUTION	NORMAL BLOOD SERUM	X-RAY BLOOD SERUM
Sum	661	496	298
Mean	13.22	9.92	5.96
Σ deviations²	200.96	181.09	203.89

Sum of deviations² = 585.94. Degrees of freedom = 3 × 49 = 147. Variance = 585.94/147 = 3.99.
Sum of the sums = 661 + 496 + 298 = 1455. Average = 1455/3 = 485.
$(661 - 485)^2 + (496 - 485)^2 + (298 - 485)^2 = 66066$
66066/50 = 1321.32
1321.32/2 = 660.66
660.66/3.99 = 165.3
Ratio of greater to lesser variance = 165.3.

Thus it will again be seen that the intergroup difference is significantly greater than the intragroup difference.

COMPARISON OF READINGS OBTAINED WITH SEEDLINGS GROWN (1) IN SHIVE SOLUTION ALONE, (2) IN SHIVE SOLUTION WITH 1 PER CENT NORMAL BLOOD SERUM, AND (3) IN SHIVE SOLUTION WITH 1 PER CENT BLOOD SERUM FROM A PSYCHOTIC PATIENT

Variance = 3.79
Sum of sums = 661 + 496 + 298 = 1455
Mean = 1455/3 = 485
$(661 - 485)^2 + (496 - 485)^2 + (298 - 485)^2 = 66066$
66066/50 = 1321.32
1321.32/2 = 660.66
660.66/3.79 = 174
Ratio of greater to lesser variance = 174.

Again it will be observed that the intergroup difference is very significantly greater than the intragroup difference.

These detailed analyses of data obtained in typical experiments with control solutions in plant physiologic saline, on the one hand, and with normal serum and blood sera obtained from patients with pemphigus, x-ray therapy, and grave psychoses, on the other, leave no doubt as to the reliability of the

phytopharmacologic technique, as practiced in this laboratory, and the positive significance of the data obtained thereby. This, of course, applies to the phytopharmacologic test as employed by an experienced worker in the field. While our statistical analysis was made on long-range experiments, i.e., experiments in which 50 seedlings were employed for each solution studied, Macht and his co-workers have discovered in actual clinical practice that reliable data regarding the phytotoxic properties of blood sera are obtained by means of a smaller number of seedlings. The routine procedure in this laboratory is to employ ten healthy seedlings for each solution and to repeat the experiment on two or three successive days. If the phytotoxic indices derived from the successive experiments vary by no more than 5 per cent, the average figure obtained has been found to be very reliable as it almost invariably agrees with the final diagnosis of the blood picture made independently by the clinical practitioner and pathologist who forward the sample for pharmacologic study.

SUMMARY

1. The Macht technique for determination of phytotoxic reactions of blood sera is described in detail.

2. Tables presenting the phytotoxic indices obtained in phytopharmacologic examination of numerous toxic, nontoxic, and atoxic blood sera are offered for use as criteria in carrying on further work of this kind.

3. A biometric analysis of data obtained in typical experiments with normal human blood serum and blood sera from persons with pemphigus, x-ray therapy, and grave mental disease is made in detail to illustrate the reliability of the phytopharmacologic test and the significance of results secured by this means.

4. The various data adduced demonstrate the utility of the phytopharmacologic test or the application of plant physiology to investigation of clinical problems.

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CLINICAL AND EXPERIMENTAL

SULFAPYRIDINE IN EXPERIMENTAL BRUCELLOSIS*

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WITH the advent of sulfapyridine in the field of therapeutics, especially in the treatment of pneumonia, a disease in which it produces such remarkable beneficial results, it was perhaps to be expected that it would be tried in the treatment of many other diseases, and to be hoped that it would show the same remarkable curative value in some of them as in pneumonia. Likewise, its ability to prevent and cure pneumococcal infections in experimental animals has been demonstrated many times by many investigators. Little has appeared in the literature so far reporting experimental results with this drug in the treatment of Brucella infections. On the contrary, many reports of the results of experimental work with regard to treating these infections with sulfanilamide are available. Many investigators have also reported various degrees of satisfactory results in the treatment of human brucellosis with sulfanilamide and its derivatives. Since the two drugs, sulfanilamide and sulfapyridine, are so closely related chemically, it is reasonable to expect them to show certain similarities in their therapeutic properties and that sulfapyridine might show even a better result in the treatment of these diseases.

Chinn[1] in a recent article reports that sulfanilamide has both preventive and curative effect on guinea pigs inoculated with *Br. abortus, Br. melitensis,* and *Br. suis.* Menefee and Poston[2] also report good results in the treatment of *Br. melitensis* var. *melitensis, abortus,* and *suis* with sulfanilamide.

To our knowledge no previous report on work with sulfapyridine in experimental Brucella infections of guinea pigs has been published.

The present report deals with results that were obtained with a group of guinea pigs after their experimental infection with the three Brucella organisms named above, as well as with the in vitro tests using the same organisms.

METHOD

The cultures used in this work were kindly supplied by Dr. I. F. Huddleson,[3] of the Michigan State College of Agriculture. We found that these organisms grew well on meat extract agar, but resulted in a more abundant growth on beef liver infusion agar. Because they did not require increased carbon dioxide tension for growth, we did not use this method in any of our cultures.

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IN VIVO TESTS

We injected intraperitoneally 56 male guinea pigs of about the same age and size and divided them into three groups. The dose of organisms given each animal was one-half of a beef liver infusion agar slant suspended in normal saline solution. Normal saline solution slightly in excess of 2 c.c. was placed in the culture tube, and the organisms were stirred into an even suspension. One cubic centimeter was injected; therefore, one culture made two doses. Of the group of 56 guinea pigs, 16 were injected with *Br. melitensis,* 16 with *Br. abortus,* and 24 with *Br. suis.* These groups were subdivided into groups as follows: four, six, two, and four for each of the *Br. melitensis* and *Br. abortus;* and five, nine, four, and six for the *Br. suis.* The groups of four, four, and five in the case of each organism were given 100 mg. of sulfapyridine daily, orally, beginning one and one-half hours after the injection. The groups six, six, and nine in each organism were given 200 mg. of the drug by mouth daily beginning six days after the injection. The groups two, two, and four were given 100 mg. of sulfapyridine subcutaneously suspended in distilled water. In the groups four, four, and six no drug was given, and these animals served as controls. The drug was given once daily in a single dose in all instances by mouth, except as indicated above. The treatment was terminated at the end of three weeks in all groups. During the three weeks' period of treatment 15 animals died; therefore, the autopsy report contained 41 animals. The oral administration of the drug was accomplished with ease by giving a broken piece of the tablet rather than the powder. The animals receiving the drug beginning one and one-half hours after the injection were for the purpose of determining its preventive value, while from the group in which the drug was begun six days after injection we hoped to determine its curative effect. At the end of the treatment period all the animals were killed except half of the groups six, six, and nine. These were kept living for twenty-one days longer to determine by comparison with the other members of this group whether there occurred a flare-up of any hidden or latent injection. During the period of treatment all animals looked fairly normal except for a small per cent that appeared sick, some of which died. The greatest mortality rate occurred in the *Br. suis* group.

At autopsy the abdominal organs were noted for obvious pathology. No sections for microscopic study were made. In some of the animals gross evidence of disease was obvious in the form of small abscesslike lesions on the surface of the liver. There was also seen in some animals tough, exudative adhesions between the intestines and liver, liver and abdominal wall and intestines. The liver and spleen in all animals were weighed and were found in most cases not to exceed the normal weight. The average weight in the treated group of the spleen was 1.19 Gm. and of the liver 29.32 Gm. In the control group the average weight of the spleen was 1.12 Gm. and of the liver 30.46 Gm.

Beef liver infusion agar cultures were made from the sectioned liver and spleen from each animal. In a few instances cultures from the heart's blood were also made, but these were not completed. Since doing this work we attended a symposium on "Brucella Infection and Hodgkin's Disease" at Duke

University Medical School, participated in by Forbus, Poston, Gunter, and Wise, who report that the primary seat of involvement in experimental guinea pig infections with these organisms is found in the lymph glands. In our animals we did not notice any lymphadenopathy.

The cultures were examined after one week's incubation at 37° C. and evidence for positiveness was made by naked eye examination and Gram-stained smears. Table I summarizes these findings.

TABLE I

IDENTIFICATION MARK OF ANIMALS	SUIS			MELITENSIS			ABORTUS		
	LIVER	SPLEEN	HEART'S BLOOD	LIVER	SPLEEN	HEART'S BLOOD	LIVER	SPLEEN	HEART'S BLOOD
Blue (100 mg.)	–*	–	–	–	+	–	–	+	–
	–	–	–	–	–	–	–	+	
	–	–		–	–		–	–	
	–	–		–	–				
Red (200 mg.)	–	–	–	–	–	–	–	–	
	–	–	–	–	–		–	–	–
	–	–					–	–	
	–	–							
Control (No drug)	–	–	+	–	–	+	+	–	–
	–	+	–	+	–	–	–	–	–
	–	+		+	–		–	+	
	–	–		–	+		–	+	
Purple (100 mg. subcutaneously)	–	–		–	–		–	–	
				–	–		–	–	
				''HOLD OVERS''					
Red (200 mg.)	+	+	+	–	–	–	–	+	+
							–	–	–

*Each check indicates negative or positive culture findings and also each check under liver column represents one guinea pig.

IN VITRO TESTS

In order to determine the bactericidal (lethal) effect of sulfapyridine when mixed in broth cultures with the organism we followed this technique:

A forty-eight-hour beef liver infusion broth culture was prepared and a portion of this diluted 1:100. One-tenth cubic centimeter of this dilution was placed in each of five flasks containing 100 c.c. of beef liver infusion broth. To the first of these 100 mg. of sulfapyridine were added, giving a concentration of the drug of 1:1,000. A sterile 100 mg. per cent solution of sulfapyridine was prepared and to the second flask 10 c.c. were added, giving a drug concentration of 1:10,000; to the third flask 1 c.c. was added, giving a concentration of 1:100,000; to the fourth flask 0.1 c.c. was added, giving a drug concentration of 1:1,000,000; no drug was added to the fifth flask which served as a control. Immediately after mixing the organisms and drug, pour plates were made, using 0.1 c.c. of the drug-organism mixture and beef liver infusion agar as the pour medium. This was repeated at intervals of ten minutes, twenty-four, forty-eight, and seventy-two hours. After seven days' incubation of the pour plates counts were made with the results shown in Table II. The counts showed that the drug in 1:1,000 and 1:10,000 concentrations had definite or complete bactericidal power against *Br. melitensis* and *Br. suis*, but these

concentrations showed only definite bactericidal or bacteristatic effect against *Br. abortus*. In the 1:100,000 and 1:1,000,000 drug concentrations some bactericidal effect was noted against all organisms.

TABLE II

PERIOD OF INCUBATION	NO DRUG	SULFAPYRIDINE 1:1,000	SULFAPYRIDINE 1:10,000	SULFAPYRIDINE 1:100,000	SULFAPYRIDINE 1:1,000,000
			Br. suis		
Immediate*					
Count	0	10	10	1,100	180
10 min.	1,310	0	0	1,330	670
24 hr.	508,880	0	0	6,350	255,889
48 hr.	11,456,200	0	0	150,821	11,456,200
72 hr.	61,858,080	0	0	3,196,000	50,105,033
			Br. abortus		
Immediate					
Count	670	3,380	2,750	3,190	2,810
10 min.	2,840	3,510	3,670	2,880	3,650
24 hr.	41,226,130	14,630	325,870	458,200	14,897,480
48 hr.	17,938,840	282,830	221,460	2,674,780	233,550
72 hr.	25,774,200	521,210	1,208,420	19,639,940	15,464,520
			Br. melitensis		
Immediate					
Count	0	0	0	0	0
10 min.	20	0	0	0	0
24 hr.	180	0	380	700	122,180
48 hr.	5,487,040	0	910	1,179,880	17,772,640
72 hr.	19,383,190	0	1,000	9,330,260	28,743,030

*These figures are actual plate counts of bacteria per 1 c.c. of the different cultures.

CONCLUSIONS

We conclude from the results of this work that sulfapyridine in treating experimental brucellosis in the method employed in these experiments showed only a slight preventive or curative effect. However, we do think that it is significant that in the group of animals receiving 200 mg. there was not a single animal showing pathologic evidence or a positive culture, except in the group that was held over for quiescent infection determination. A glance at Table I will show that two groups of these were positive. This would seem to indicate that the infection was being held under control by the drug and underwent a flare-up after it was discontinued. We are aware of the fact that sulfapyridine, as given in this experiment, was not administered as frequently as is desired, yet the size of the largest dose when based on body weight, which is the usual way of computing dosage, is about eight to ten times larger than the average size for human beings, and this might in some way compensate for less frequent administration.

Armstrong and Muirhead[4] recently reported that in a large series of guinea pigs 100 mg. of sulfapyridine orally every four hours maintained blood drug concentration at about the 7 mg. per cent level. Further, they report that 50 mg. orally every four hours did not keep the level up to what is generally considered to be optimum, while 200 mg. every four hours kept the level above the optimum of about 7 mg. per cent. On the basis of this work, we have reason to believe that after the drug was given in our animals the blood concentration reached about a 6 mg. per cent to a 15 mg. per cent level and then gradually fell before the next dose.

In our in vivo tests the drug seemed to show best results in *Br. suis* infections and poorest results in *Br. abortus*. In the animals held over for twenty-one days longer without treatment for evidence of latent or hidden infection, we found heavy infections in both *Br. suis* and *Br. abortus* animals and negative pathologic and cultural evidence with *Br. melitensis* animals.

The in vitro tests showed that in the 1:1,000 and 1:10,000 concentrations against *Br. suis* the drug resulted in complete destruction of the organism. In the 1:100,000 concentration against this organism definite bactericidal effect was noted, while the 1:1,000,000 dilution showed only feeble destructive power. These findings are in agreement with the in vivo findings, since it was in *Br. suis* infection that best results were noted. Against *Br. melitensis* the 1:1,000 drug concentration showed complete bactericidal power; the 1:10,000 nearly complete killing power; the 1:100,000 concentration definite destructive power; and the 1:1,000,000 concentration showed no killing power. The effect of the drug against *Br. abortus* was different when compared with the other two organisms. This organism was far more resistant; the 1:1,000 concentration showed a definite bactericidal effect only and the remaining dilutions showed less effect as the drug concentrations were reduced. In other words, the drug had far less killing power against *Br. abortus* when compared with its killing power against *Br. suis* and *Br. melitensis*. Therefore, it appears that in the therapy of these infections the drug is more potent against *Br. suis* infection, less potent against *Br. melitensis*, and least potent against *Br. abortus*.

SUMMARY

1. Three groups of normal healthy guinea pigs were each injected with *Br. suis*, *Br. melitensis*, and *Br. abortus*.

2. The dose of organisms used was one-half of a beef liver infusion agar slant culture.

3. Each main group of injected animals was divided into four subgroups and each subgroup was treated as follows: one subgroup was given 100 mg. of sulfapyridine daily by mouth, beginning one and one-half hours after injection; one was given 200 mg. of the drug daily by mouth, beginning six days after injection; one was given 100 mg. of the drug subcutaneously, beginning six days after injection; and one was not given any drug and served as a control group.

4. All treatment was terminated twenty-one days after injection.

5. At the end of this treatment period all animals were autopsied, except half of each of the groups receiving 200 mg. These were held for twenty-one days longer to determine whether a flare-up of the infection occurred during the no drug period.

6. The drug used for the in vitro tests was a 100 mg. per cent solution in distilled water. This was suggested by Dr. Robert Hoyt,[5] of the University of Minnesota, who reported that he found a 100 mg. per cent solution satisfactory for general use.

7. The medium used throughout was beef liver infusion broth and agar. This medium was selected because it seems to possess certain nutritional substances promoting growth of these organisms.

8. The cultures used in these experiments were obtained from Dr. Huddleson, of Michigan State College.

9. We feel that the drug in the method used does not control or cure Brucella infections in guinea pigs, in spite of the fact that based on body weight the amount given was far greater than the usual (80 grains a day) therapeutic amounts given human beings. We believe that the drug has curative value against at least two of the types of Brucella infection *(Br. suis* and *Br. melitensis)* when given frequently enough to maintain a constant blood level.

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CHANGES IN THE SEDIMENTATION RATE AND NONFILAMENT-FILAMENT RATIO FOLLOWING CLINICAL IMPROVEMENT IN PATIENTS WITH LOW-GRADE CHRONIC ILLNESS

A Statistical Analysis of 323 Cases

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IN A STUDY of patients with low-grade chronic illness, in which chronic infection appeared to play an important part,[1] it was reported that (a) practically all patients showed an increased nonfilament-filament ratio; (b) more than 80 per cent showed an increased sedimentation rate; (c) the nonfilament-filament ratio and the sedimentation rate were higher in patients with severe symptoms than in those with moderate or mild symptoms; and (d) in acute exacerbations the nonfilament-filament ratio was increased relatively more than was the sedimentation rate. The present study was made to determine the character and extent of the changes in the nonfilament-filament ratio and the sedimentation rate which were associated with clinical improvement or recovery in a similar group of patients.

CLINICAL DATA

Studies of the blood were made on 323 patients with chronic or frequently recurring symptoms, many of which were probably secondary to low-grade chronic infection. Patients were grouped according to severity of illness, as having mild or recurrent, moderately severe, or severe symptoms. An additional group included patients with more or less acutely increased symptoms. Detailed analysis of a similar series of patients as to age, sex, and clinical diagnosis have been reported.[1]

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Following definite clinical improvement, whether spontaneous or following treatment, the blood studies were repeated. Improved patients were grouped as symptom free, or as having mild or moderate symptoms. Patients were also grouped according to the degree of improvement, from mild to symptom free, from moderate to mild, or from severe to moderate being considered as one stage; from moderate to symptom free or from severe to mild as two stages; and from severe to symptom free as three stages of improvement. The group of patients with acutely exacerbated symptoms was not included in this analysis because the nonfilament-filament ratio and the sedimentation rate did not conform to relationships found in other groups.[1]

METHODS

Sedimentation rate determinations and complete blood counts were made on all patients. Except for nonfilament-filament ratios, hematologic data will not be discussed because the changes were inconstant or infrequent. In making differential counts neutrophiles in which two or more lobes were united only by a filament of chromatin were recorded as filamented cells, others were classified as nonfilamented. The ratio of nonfilamented to filamented neutrophiles (referred to as nonfilament-filament ratio) was expressed in percentage, i.e., $\text{nonfilament-filament ratio} = \frac{\text{nonfilamented cells} \times 100}{\text{filamented cells}}$.[1]

In determining the sedimentation rate oxalated blood was drawn up to the 200 mm. mark on a Westergren tube. At the end of one hour the height of the column of clear fluid was recorded in millimeters.[1]

TABLE I

CHANGES IN THE SEDIMENTATION RATE AND NONFILAMENT-FILAMENT RATIO IN PATIENTS SHOWING CLINICAL IMPROVEMENT

FINDINGS	PATIENTS	RANGE	MEAN	STANDARD DEVIATION	STANDARD ERROR
Original					
Nonfilament-filament ratio	299	10-100	29.75	14.34	0.83
Sedimentation rate	200	2-99 mm.	21.15	18.05	1.275
Following improvement					
Nonfilament-filament ratio	299	4-24	10.26	5.18	0.3
Sedimentation rate	200	1-35 mm.	6.425	5.85	0.41

Table I shows a comparison of the sedimentation rates and nonfilament-filament ratios, both originally and after definite improvement. The mean nonfilament-filament ratio of 299 patients was 29.7 originally and 10.3 following definite improvement. Although some of the patients showed higher ratios after improvement than did others originally, the difference between the mean values before and after improvement was 18.3 times its standard error. The mean sedimentation rate of 200 patients was 21.15 mm. originally and 6.42 mm. following improvement. The difference between the two mean values was 11.4 times its standard error.

As noted previously, patients were classified according to the number of stages of improvement (Table II). The reduction in the mean nonfilament-filament ratio was 40.7 in patients with three stages of improvement, 22.4 in

those with two stages, and 12.0 in those with one stage. The mean sedimentation rate was reduced 35.7 mm. in those with three stages, 18.4 mm. in those with two stages, and 7.5 mm. in those with one stage of improvement.

The relationship between improvement in clinical and laboratory findings was less constant in patients with only one stage of improvement, two patients in this group showing slightly higher nonfilament-filament ratios after improvement, and seven showing either no change or a slight increase in the sedimentation rate.

The differences between the mean gains shown by the various groups were all statistically significant.

TABLE II

RELATIONSHIP BETWEEN CHANGES IN THE SEDIMENTATION RATE AND NONFILAMENT-FILAMENT RATIO AND THE DEGREE OF CLINICAL IMPROVEMENT

DEGREE OF IMPROVEMENT	PATIENTS	RANGE	CHANGE IN VALUES		
			MEAN	STANDARD DEVIATION	STANDARD ERROR
1 stage					
Nonfilament-filament ratio	137	+3 to -29	-11.98	4.97	0.425
Sedimentation rate	94	+3 to -29 mm.	- 7.84	6.94	0.715
2 stages					
Nonfilament-filament ratio	113	-1 to -49	-22.38	9.33	0.88
Sedimentation rate	69	0 to -69 mm.	-18.44	13.88	1.67
3 stages					
Nonfilament-filament ratio	39	-17 to -75	-40.69	12.18	1.95
Sedimentation rate	26	- 5 to -94	-35.69	22.1	4.34

CONCLUSIONS

Clinical improvement in patients with low-grade chronic illness is accompanied by statistically significant improvement in the sedimentation rate and nonfilament-filament ratio.

The degree of improvement in the sedimentation rate and non-filament-filament ratio varies significantly with the degree of clinical improvement.

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113 W. CHESTNUT HILL AVE.

BRONCHIECTASIS ASSOCIATED WITH MONILIA SIMULATING PULMONARY TUBERCULOSIS*

A Clinical-Pathologic Study

E. Robert Wiese, M.D., White Haven, Pa., and E. W. Bixby, M.D., Wilkes-Barre, Pa.

BRONCHIECTASIS, because of the problems relative to etiology, prognosis, pathology, and treatment, has been a subject of interest to medical men from the time of its first description by Laënnec to the present day. Regarded as a comparatively rare disease when only autopsy material was available, the modern use of radio-opaque substance has shown the condition to be more prevalent than formerly believed, and both medical and surgical interest has correspondingly increased. Without the use of such radio-opaque substances the diagnosis is not easy, and its chronicity, complications, and verisimilitude to other pulmonary diseases has frequently led to errors in diagnosis. Infection of the bronchiectatic dilations by nearly all the microorganisms found in pulmonary disease is almost a logical sequence of events and has been the subject of several papers and discussions by many men. The following is submitted as a peculiar infection in a well-defined case of bronchiectasis, erroneously diagnosed as pulmonary tuberculosis.

E. W., a white, unmarried woman of 30 years, entered the White Haven Sanatorium on April 4, 1938, with a diagnosis of pulmonary tuberculosis in the service of one of us (E. W. B.). Except for the fact that her father had died of carcinoma of the stomach in 1912, the family history was negative. Her past history included an attack of influenza in 1919, two attacks of pleurisy without effusion and bronchitis previous to 1936, and numerous colds. There was no history of whooping cough or of any contact with tuberculosis. The present illness began in April, 1936, with a pulmonary hemorrhage of 8 ounces. After approximately three months' rest at home, the patient went to a private sanatorium from July to October, 1936, but returned to work as a stenographer on November 1 of that same year. She did well until March 7, 1938, when she had a second severe and protracted hemorrhage of approximately 24 ounces. A few days afterwards x-rays of the chest were taken and sputum tests were made. Although no tubercle bacilli were found in the sputum, the patient was diagnosed as having pulmonary tuberculosis on the basis of the history, clinical evidence, and x-ray findings—a perfectly reasonable assumption. Her account of the cough of which she complained was interesting. She was quite definite in her statement that the many "colds" with which she was afflicted began in April, 1935, a year before her first hemorrhage. She had coughed ever since that date; her sputum was an odorless, yellowish green fluid, and varied in amount from 1 to 4 ounces daily. Despite many tests, no tubercle bacilli were demonstrated.

*From the Medical Department of the White Haven Sanatorium, White Haven, Pa.
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Upon admission the patient was a rather sickly appearing pale woman. The right side of her chest, somewhat smaller than the left, did not expand freely. The quality of the percussion note increased from impairment in the upper part to distinct dullness at the base where there was a suggestion of a pleural rub. The whispered voice was increased throughout the entire right side of the chest; the breath sounds were bronchial in character and coarse râles, especially at the base, were present. The left side of the chest was a trifle full and expansion was good; the percussion note was impaired in the upper part where the whispered voice was somewhat increased and the breath sounds were broncho-vesicular in character. Otherwise the left side of the chest was negative. The cardiac point of maximum impulse was found in the fifth intercostal space inside the midclavicular line. No cardiac murmurs were heard. The fingers were not clubbed, nor were the nails curved. The blood pressure upon admission was 116/80, the red blood cell count was 4,400,000, the white blood cell count was 10,200, of which 68 per cent were polymorphonuclear cells and 30 per cent lymphocytes. The hemoglobin was 80 per cent and the Wassermann test was negative. Repeated sputum examinations were, and continued to be, negative for Koch's bacillus. The urinalysis was negative. Examination by Dr. Joseph C. Donnelly, of Philadelphia, revealed infection of the left maxillary sinus. The x-ray report of April 15, 1938, read as follows:

> "The left lung is essentially normal. The shadows over the lower portion of the right lung are evenly increased in density, and there is a distinct atrophy of the right side of the chest indicative of a bronchial occlusion. The etiological factor is not clear, but if the sputum be negative, I would be suspicious of a benign growth. A pneumonography and a bronchoscopic examination may throw some further light on the condition." (Fig. 1.)

The last sentence in this report strengthened our determination to use some radio-opaque substance to determine the presence or absence of a condition we had previously suspected from a study of the history, clinical findings, and x-ray films on the case. Lipiodol was introduced into the trachea by the intercricothyroid route on May 16, 1938, and the x-ray report read as follows:

> "The iodized oil has entered the bronchial branches of the right lower and I believe the right middle lobe. It has collected in pools in cylindrical dilatations along the bronchi. The changes are those of a bronchiectasis involving particularly the lower lobe and I suspect there is also some involvement of the middle lobe." (Fig. 2 and Fig. 3.)

The patient's temperature, 101° F. upon admission to the White Haven Sanatorium, became normal under the influence of bed rest. Her pulse, 108 when the patient was first seen, also became normal under a like treatment. She weighed 94 pounds when she first came to the White Haven Sanatorium and gained 25 pounds in the next six months (119 pounds).

Even before the diagnosis of bronchiectasis was thus made, a number of yeastlike cells were discovered in the sputum during the continuous search for the tubercle bacilli. A culture medium of glucose, powdered raw potato, peptone, and agar was inoculated with the sputum and cultivation was carried on at room temperature. An organism which closely resembled *Monilia albicans* (Castellani) was identified by morphologic, structural, and cultural characteristics. The cells, either round or somewhat oval in shape, varied in size; the mycelium was septate and conidia were formed both by budding near the joints and abstriction at the ends of the hyphae. The culture characteristics were tested in a strained medium made up of peptone, powdered raw potato, and water; to this were added in different fermentation tubes dextrose, maltose, lactose, galactose, levulose, dextrin, and monnite—a separate sugar to each tube; aqueous acid fuchsin (0.5 per cent) was used to

determine the formation of acid during the growth of the fungus in these tubes during the next two or three weeks at room temperature. Gas and acid formation were observed in the tubes containing dextrose, levulose, maltose, and galactose in three successive cultures during the next ten weeks. The organism was identified as *Monilia albicans* (Castellani).

Our inability to discover any evidence of thrush in the mouth or pharynx was confirmed by Dr. M. J. Hess (D.D.S.) of White Haven, who cleared the mouth of all objectionable or suspicious elements. The gums were thoroughly healed before work was begun upon the sputum, and the patient was instructed to use a strong mouth wash before expectorating—a precept which we are certain she rigidly followed.

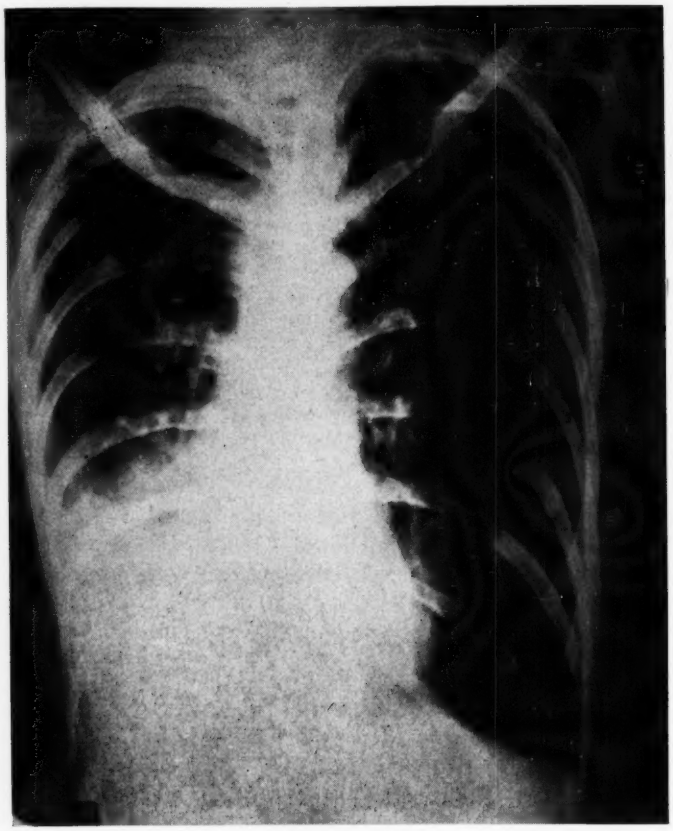

Fig. 1.

COMMENTS

Because of the generally accepted opinion of medical men that the disease is one seen only by those engaged in tropical medicine—and possibly also because of the little space devoted to the subject in even the best textbooks on diseases of the lung—fungus infection of the respiratory tract has received little attention to the present time from the medical profession of the temperate zone. A growing literature within the past fifteen years, however, has aroused interest in the disease, and has placed among diagnostic possibilities a condition that may not only simulate especially the more chronic diseases of the lung, but may affect the prognosis when seen as a complication and may even cause death.

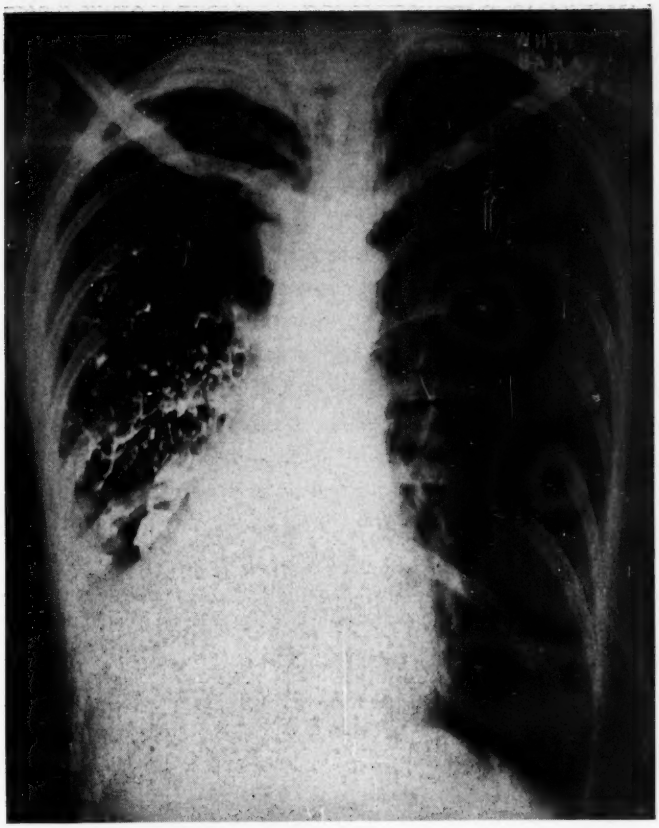

Fig. 2.

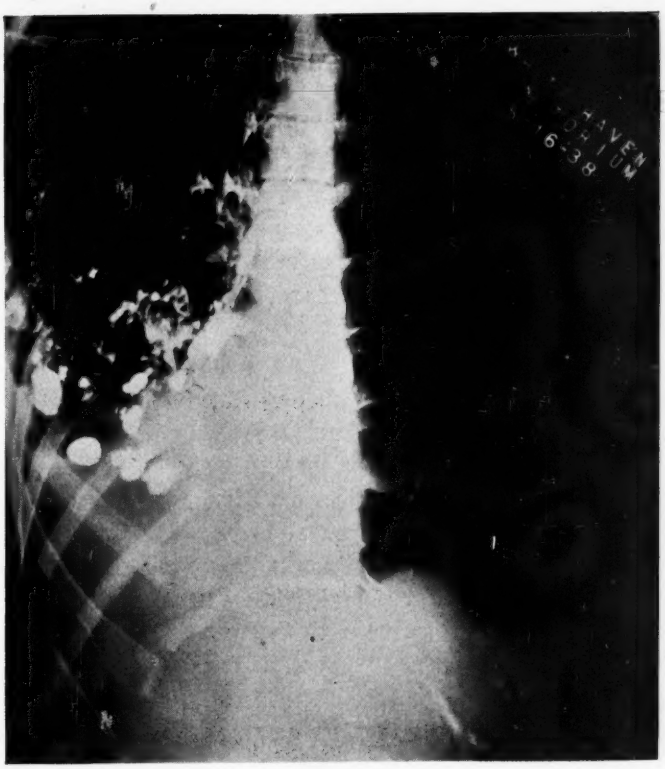

Fig. 3.

Classification of the microorganisms included in the subject of mycology is in a state of almost hopeless confusion. Almost any one of the authorities seems to mention the classification proposed by another authority only to differ from it and to propose his own classification in its stead. One authority, indeed, goes so far as to suggest the scrapping of all classifications thus far proposed and to include all fungi under the general heading of blastomyces. Nor do they agree as to methods. Castellani, for example, although admitting the occasional failure of the procedure, lays stress upon the ability of the microorganisms to ferment the various sugars and to produce acid in the different solutions as a means of identification. Dodge, on the other hand, scouts the idea and seems to rely solely upon the morphology of the microorganism in question. Even the biological relationship between monilia, oidia, torula, and yeasts is not a settled thing among various authors, although almost without exception they classify the monilia among fungi imperfecta.

Irrespective of classification, however, all authorities agree that fungus infection of the lung is possible and divide the cases into three types. In the mild type of infection the general condition of the patient is not much impaired. There is little or no fever; the sputum is generally scanty and mucopurulent in character; a few râles may be heard but usually examination of the chest is negative. In the intermediate type there is evidence of a mild catarrhal bronchitis; a slight fever is usually found; the cough, differing in character in different patients, is usually paroxysmal and, as a rule, is most severe in the morning and the evening. Dyspnea is not infrequently one of the symptoms. In the severe type the patient loses weight, the fever is hectic, the sputum is rather profuse, and at times hemorrhagic; night sweats and a marked anemia are very often found. Chest examination reveals areas of dullness, fine râles, and evidences of pleuritis; in short, the picture of the severe type closely resembles that of pulmonary tuberculosis, for which it may be readily mistaken. The prognosis naturally varies with the virulence of the infection. The mild type may clear up spontaneously or after several weeks or months may develop into the intermediate or the more severe type; it usually yields to treatment. The intermediate type is more stubborn, and the response to treatment is often slow. The therapy must be continued, as such cases may readily develop into the third type. The severe type is often difficult to combat, and cases of death due to mycotic infection of the lung are not wanting (Stovall and Greeley, Warr).

The x-ray film in this disease also closely resembles that of pulmonary tuberculosis. There may be peribronchial thickening, nodules, infiltration, pleural thickening, areas of consolidation, and even fibrosis shown in the film (Bakst, Hazard, and Foley). The apices usually remain clear even in the most advanced cases, the disease being essentially a basal one (Balog and Grossi). Little is known of the pathologic findings in man, as few autopsies have been reported. In the case cited (Warr) both right and left lungs were adherent to the chest wall; there were numerous cavities on each side, and the lower lungs were consolidated and easily torn. The cut surfaces were glistening, shiny, and translucent. Both lungs were greatly reduced in size with little air-bearing space in either. Potassium iodide seems of value in the treatment of the disease,

especially of the first and second types. Castellani advocates the administration of 15 grains of potassium iodide in milk or water three or four times a day over a period of three to four weeks. Other medication has also been lauded. Balog and Grossi favor the use of insulin, Stovall and Greeley gave gentian violet intravenously. Thymol also has its advocates. Vaccine treatment has been proposed, and Kotkis, Wachowiak, and Fleisher, for example, reported complete disappearance of symptoms in one case and marked improvement in another. Balog and Grossi are of the opinion that vaccine treatment is the sole therapy capable of restoring the injured myocardium in moniliasis. Craik reports the successful use of alkalies, adrenalin, and potassium iodide. The roentgen ray as a therapeutic means has been used by Schmidt and Howe. Warr classes alkalies and iodide by mouth, and autogenous vaccines and ultraviolet rays as energy providers.

SUMMARY

A case of bronchiectasis in conjunction with *Monilia albicans* has been reported, together with a brief discussion on mycotic infection of the respiratory tract. We are of the opinion that mycotic infection of the respiratory tract is of more frequent occurrence than is generally believed, and that careful search of cases closely simulating pulmonary tuberculosis in the absence of tubercle bacilli in the sputum will reveal this condition, especially where the lesion is of the basal type.

In conclusion, may we say that we do not wish to leave the impression that we believe that Monilia was in any way responsible for the bronchiectasis, but rather that the bronchiectasis offered a very favorable soil for the growth of the fungus when once it had reached the lung. Another point worthy of note is that bronchiectasis and moniliasis, both pre-eminently basal affections of the lung, individually closely simulate basal pulmonary tuberculosis. The combination of the two diseases certainly more than doubles the resemblance. The fact that the specimens were not obtained directly with the bronchoscope need not militate against the results as all possible means were taken to eliminate oral contaminations. If this failure is a fault, then it is one shared by all cases we found in literature.

Comments by Dr. E. W. Bixby: The history of the case to date is also interesting. After it had been determined by all the usual tests (including animal inoculation) that the patient was not suffering from pulmonary tuberculosis, she was removed from the White Haven Sanatorium on November 19, 1938, to a hospital in Wilkes-Barre, where her accessory nasal sinuses were thoroughly drained. Ten bronchoscopic drainages were also done at the same hospital. She was then transferred to the Pennsylvania Hospital, Philadelphia, in the latter part of June, 1939, where Dr. John B. Flick removed the lower lobe of the right lung. Because of the many and dense adhesions present, the operation was a very prolonged and difficult task, but the patient withstood the operation well and made an uneventful recovery until August 15, 1939, at which time she developed an empyema. Closed drainage was instituted and a recent letter from Doctor Flick reports the patient in good condition.

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PRIMARY MALIGNANT TUMORS OF THE SPLEEN WITH REPORT OF A CASE OF LYMPHOSARCOMA*

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THE infrequency of primary malignant tumors of the spleen affords sufficient reason for reporting single cases as they occur in the practice of individual surgeons, or as they are found on the autopsy table by pathologists. Examination of the literature shows that very few surgeons have removed more than one tumor of this kind; and in this connection it may be stated that the case here reported is the only one to be found in the records of the Jefferson Medical College Hospital.

In 1929 Tasker Howard,[1] of Brooklyn, found 115 cases recorded in the literature and added another case that had occurred in his own practice. Since the publication of Howard's paper, I have succeeded in finding 23 more evidently authentic cases[2-22] which, with the one here reported, brings the total number of cases to 140.

Because of the rarity with which these cases occur, and also because of the complex structure of the spleen, classification of the tumors has been difficult. Growths called carcinoma have been reported, but the histologic structure of the spleen renders the development of a primary epithelial growth impossible, unless it is of the fetal inclusion type.

Various names have been applied to tumors of the connective tissue type, and even at the present time it would seem that a definite classification has not been firmly established. For instance, as late as 1935, Stevenin[17] and his associates studied a tumor, which, they stated, could not be placed in any of the classifications previously made. Because of its resemblance to undifferentiated reticular sarcoma of bone marrow, they finally decided to call it undifferentiated reticular sarcoma.

Without reviewing the various classifications, it may be stated that a primary malignant neoplasm may take origin from the connective tissue in the splenic capsule; from the reticular stroma of the spleen; from the endothelial

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cells in the walls of the blood vessels; from the reticulo-endothelial cells lining the splenic sinuses; and from the lymphoid tissue surrounding the arterial vessels as an interrupted sheath, known as the splenic nodules or Malpighian corpuscles. The endothelial and lymphoid types are apparently the most common, the latter being termed lymphosarcoma. Vascular growths known as malignant hemangioma, hemangiosarcoma, and hemangio-endothelioma are also of relatively frequent occurrence.

The case that I am now reporting is that of a man, who at the time I operated upon him, January 5, 1928, was 58 years old. His history prior to the development of the splenic tumor is not without interest, in that it shows a susceptibility to disease of the reticulo-endothelial system. On two occasions, first in 1909 and again in 1921, enlarged lymph nodes were removed from the inguinal and cervical regions, respectively. In the interval of twelve years between the two operations, and also after the second operation, no evidence of enlarged lymph nodes was noticed in any part of the body.

Unfortunately, no record of microscopic examination of the inguinal nodes removed in 1909 was obtainable. Dr. Baxter L. Crawford, who examined the discrete cervical nodes removed in 1921, stated that the histologic picture was one of malignancy rather than of chronic inflammation. The possibility of lymphogranuloma was suggested, but it was definitely stated that the lesion was not typical.

Late in December, 1927, the patient consulted me again, complaining of digestive disturbance, weakness, and high abdominal pain. He stated that approximately six weeks before his visit, he noticed a swelling, which had increased rapidly in size, in the left part of the abdomen below the edge of the ribs. He also stated that he had lost several pounds in weight during the preceding month.

Examination revealed a large left-sided abdominal mass, distinctly nodular, and plainly splenic in origin. There was no fever, and blood examination, with the exception of a slight anemia of the secondary type, failed to show any abnormality. X-ray examination showed nothing significant, except the shadow of the splenic mass.

Splenectomy was performed on January 5, 1928, in the Jefferson Medical College Hospital. The tumorous spleen was very adherent to the diaphragm and had enlarged so much in the direction of the stomach that it had obliterated the space between the folds of the gastrosplenic omentum. It was, however, removed without any especial difficulty.

The pathologic report submitted by Dr. Baxter L. Crawford was as follows:

> "Specimen consists of a spleen which measures 18 by 13 by 8 cm. and weighs 755 Gm. The external surface of the greater part of the spleen is nodular, and the nodules vary in size, and are grayish red in color in sharp contrast to the red color of the splenic tissue. These nodular areas are extremely soft and in some places seem to fluctuate. On section it is seen that the greater part of the splenic pulp has been replaced by nodules of soft gray, homogeneous tissue, which are discrete and sharply defined at the margins. They do not appear to have invaded the splenic capsule. In the larger nodules the tissue is soft, yellowish gray in color, and large areas show necrosis. The small nodules are sharply defined, pearly white in color and soft in consistency.
>
> "Formalin and Zenker's fixations. Routine technique. Histology: Sections show the tumor masses to be composed of cells which vary somewhat in size and shape, but which for the most part are round with rather large, deeply staining nuclei and with a small amount of fibrous tissue stroma. There are numerous small blood vessels in this stroma. The margins of the tumor are sharply circumscribed, and although there is a condensation of the tissue, no definite capsular formation is observed.

"In the adjoining splenic tissue the follicles are numerous, large, and irregular in shape. Many possess large germinal centers. There seems to be a considerable proliferation of the cells of the follicles. The pulp of the spleen appears practically normal.

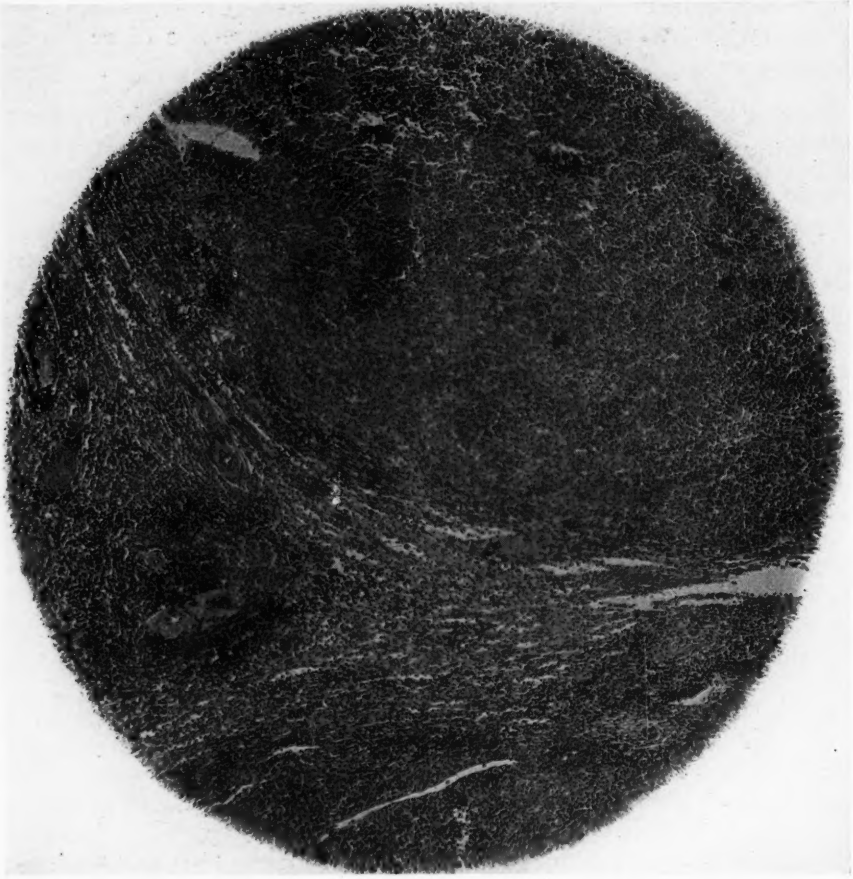

Fig. 1.—Photomicrograph of a section of the spleen (×50) showing edge of tumor; the tumor is above, the normal splenic tissue below and to the left. Observe also slight invasion of the spleen along the inferior portion of the section.

"There is no doubt that the lesion in the spleen is a malignant neoplasm, but there is some question as to the origin and type of the cell forming the growth. Because of the multiplicity of the tumors and the marked hyperplasia of the follicles, it is considered to be of lymphoid cell origin, even though in the tumor masses the cells are not identical as to the size and lymphoid type of cell.

"In the larger nodules there is extensive necrosis of the tissue.

"Sections were also stained to demonstrate the reticulum, but nothing unusual was shown in that structure.

"Diagnosis: Lymphosarcoma of the spleen" (Fig. 1).

The patient made a good postoperative recovery and went back to work within a few weeks. He remained in fairly good health until autumn. In November he began to complain of weakness, pain in the thorax, and some difficulty in urinating, and his gait became unsteady. These symptoms increased rapidly, and on December 30 he was read-

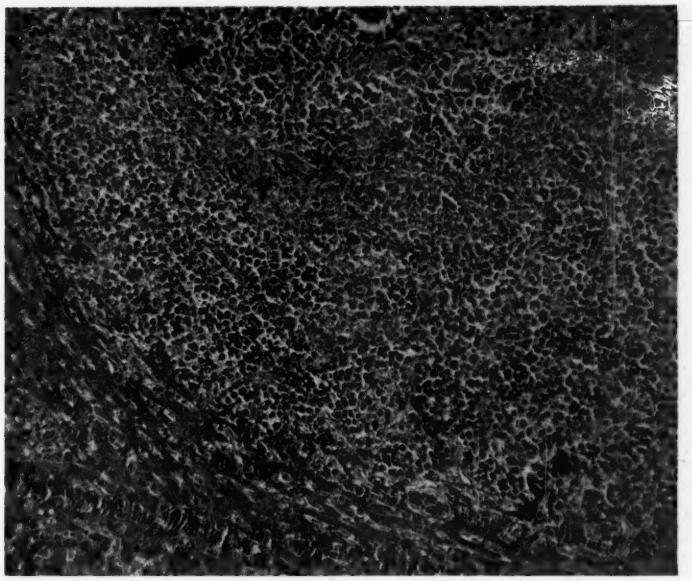

Fig. 2.—Photomicrograph of a section of the liver (×100) showing invasion by the tumor; the tumor is above and to the right, the normal liver tissue below and to the left.

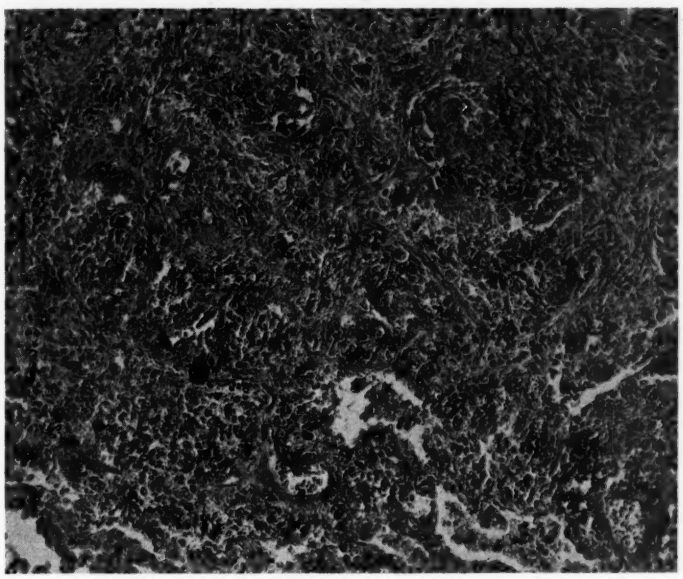

Fig. 3.—Photomicrograph of a section of the lung (×100) showing generalized invasion by the tumor.

mitted to the hospital. At that time he had complete retention of urine and had to be catheterized. By January 15, 1929, there was complete paralysis of the lower extremities and loss of sensation from the soles of the feet to the level of the umbilicus. The paralysis was accompanied by loss of control of both bladder and rectum and was soon followed by the development of gangrenous areas over the sacrum and on the lower extremities. Death occurred on February 18, 1929.

Autopsy was performed the same day by Dr. Crawford. Abridging his report, the following summary may be made: In the liver innumerable grayish nodules of variable

size and consistency were found (Fig. 2). There were also three similar nodules in the left lung (Fig. 3), and one in the cortex of the left kidney (Fig. 4). When the spinal cord was removed, a mass of firm grayish tissue was seen surrounding its lower thoracic and upper lumbar portions (Fig. 5).

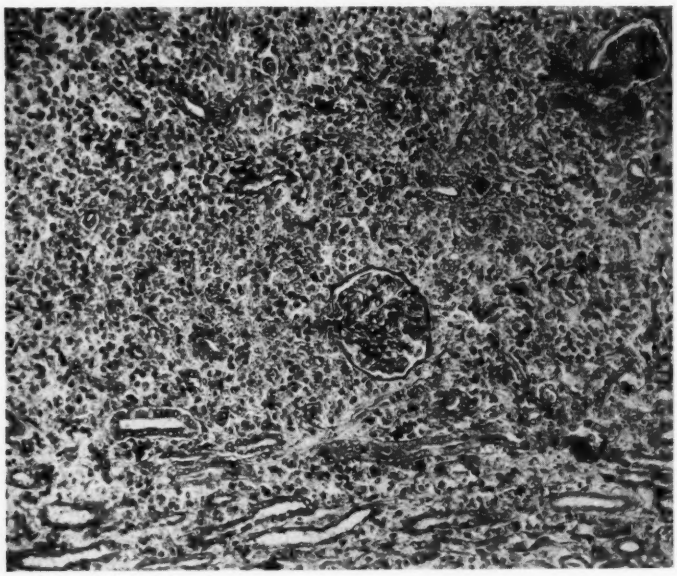

Fig. 4.—Photomicrograph of a section of the kidney (×100) showing invasion by the tumor, especially above and to the left.

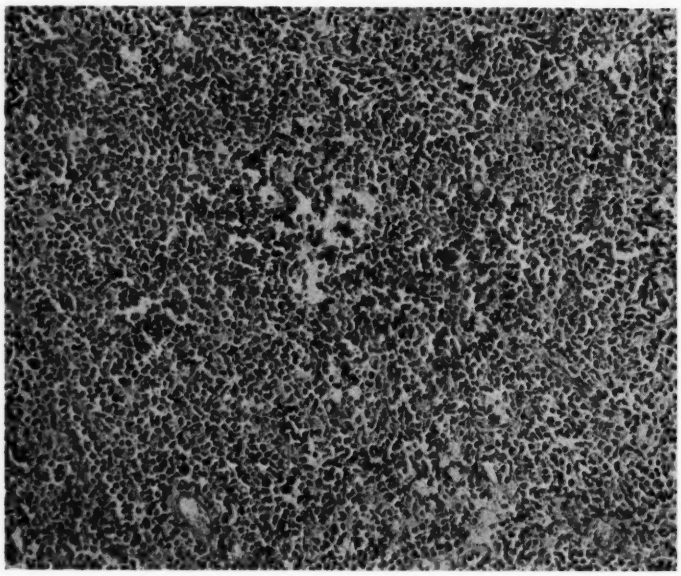

Fig. 5.—Photomicrograph of a section of the metastatic growth removed from the spinal cord (×100) showing its cellular structure.

The histologic structure of nodules removed from all three of these organs, and that of the neoplastic tissue dissected from the spinal cord, was quite similar to the histologic structure of the splenic tumor previously examined. Therefore, a diagnosis of multiple metastatic sarcoma was made.

It is noteworthy that there was no involvement of lymph nodes in any part of the body. A few of the mesenteric and retroperitoneal nodes were slightly enlarged, but microscopic examination showed only hyperplastic changes.

With regard to early symptomatology, it may be stated that malignant tumors of the spleen usually grow rapidly and that they are characterized by local pain, tenderness to pressure, and gastrointestinal disturbances. Very rapid loss of weight has occurred in some cases.

Changes in the blood are inconstant, but Howard,[1] Frank,[13] and McNee,[15] making independent observations, have called attention to the fact that in lymphosarcoma the picture may simulate that of pernicious anemia. In some cases, however, slight secondary anemia has been the only abnormality detected until late in the course of the disease; and even in some far-advanced cases the changes have been insignificant.

The growth may invade adjacent structures by direct extension and give rise to serious complications. Thus, fatal peritonitis has followed perforation of the colon caused by neoplastic invasion of that structure. The pleura has also been involved by extension of the growth through the diaphragm.

Metastases will affect the clinical picture according to the site in which they occur. For example, in the case of my patient, pressure upon the spinal cord produced a transverse myelitis, which accounted for the paralysis of the lower half of the body, with its accompanying urinary dysfunction and infection, and the development of gangrene over different areas of the paralyzed region.

From the surgeon's standpoint, malignant tumors of the spleen will require differentiation principally from benign tumors, cysts, and abscesses. Infarction without abscess formation should also be taken into consideration. It would seem that the majority of these tumors are nodular, but others, particularly some of the endotheliomas, are smooth. Absence of the normal notches along the anterior border of an enlarged spleen should arouse suspicion of tumor formation.

The prognosis is bad. In the 23 cases that I have collected from the literature, splenectomy was performed in 15. In two other instances the condition was found to be inoperable when the abdomen of the patient was opened. One of the patients died a few hours after operation; the other lived for four and one-half months. Of the nine who survived splenectomy, five are known to have died of metastases at periods varying from a few weeks to eighteen months after their discharge from the hospital. In three of the five remaining cases only a few months had elapsed between operation and publication of the case reports, so that a definite statement as to the ultimate result could not be made. The fourth patient, who had remained free from recurrence for nearly four years, developed a mass in the left flank, but it was questionable whether the lesion was metastatic sarcoma or primary carcinoma of the colon. The patient refused operation. The fifth patient was well five and one-half years after operation. The case was one of hemangio-endothelioma. Death occurred in the six cases in which operation was not undertaken.

Some pathologists believe lymphosarcoma to be part of a generalized malignant process, which will attack other structures in the reticulo-endothelial sys-

tem simultaneously with, or subsequently to, its beginning in the spleen. Furthermore, endothelial tumors have been followed by metastasis in a very short time after their removal. In a few cases, however, including both types of tumor, there has not been any reappearance of the disease for several years after extirpation of the spleen; consequently, splenectomy is advisable, provided that there are no signs of involvement of other organs when the patient first came under observation.

I am greatly indebted to Dr. Baxter L. Crawford and Dr. Carl J. Bucher, of the Pathological Department of the Jefferson Medical College and Hospital, for their cooperation in the study of the case herein reported.

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1117 SPRUCE STREET

A COMPARISON OF THE HINTON, KAHN, KLINE, AND MAZZINI TESTS FOR SYPHILIS*

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IT HAS been the practice of this laboratory to perform the Hinton, Kahn standard, and Kline diagnostic tests on all blood specimens submitteed for serologic examinations. The specificity and sensitivity of these tests have been established by many comparative studies and by several nationwide evaluation studies.

Mazzini[1] has recently described a slide test for syphilis which is claimed to be high in sensitivity and specificity, and which has many desirable features from the standpoint of performance. Giordano[2] and his co-workers have confirmed Mazzini's findings.

Since it appeared that the Mazzini test might be a valuable addition to our present procedure, this investigation was made to determine what agreement we might secure with the four tests.

PROCEDURE

When specimens were received, the sera were separated and inactivated in an electrically controlled water bath at 56° C. for thirty minutes. The Hinton, Kahn standard, Kline diagnostic, and Mazzini tests were employed in the examination of each specimen. The Hinton and Kahn antigens were prepared in this laboratory and were checked against "standard" antigens for sensitivity and specificity; the Kline antigen was purchased from LaMotte and the Mazzini antigen was furnished by Mr. L. Y. Mazzini. In the performance of the tests, the technique of the originators was strictly followed. The results are given in Tables I and II.

DISCUSSION

Tables I and II show a complete agreement of all four tests in 2,818 sera (93.93 per cent), and a relative agreement in 2,858 (95.26 per cent). In an earlier study[3] we reported that the Eagle, Ide, Kahn, Kline, and Laughlen tests agreed in 98.5 to 99.5 per cent of the sera examined. Lack of agreement was noted chiefly in sera which gave doubtful or weakly positive reactions.

In this study agreement was slightly lower than in our comparison of 1937. Lack of agreement usually occurred in sera which gave doubtful or weakly positive reactions. In several cases, however, one or more of the tests gave strongly positive reactions, while the others gave negative or doubtful ones.

*From the Arizona State Laboratory, Tucson.
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TABLE I

COMPLETE AGREEMENT OF HINTON, KAHN, KLINE, AND MAZZINI TESTS

	NUMBER OF SPECIMENS	PER CENT
All negative	2,184	72.80
All doubtful	17	0.56
All weakly positive	309	10.30
All strongly positive	308	10.27
Total	2,818	93.93
Results not agreeing	182	6.07
Complete total	3,000	100.00

TABLE II

RELATIVE AGREEMENT OF TESTS

	NUMBER OF SPECIMENS	PER CENT
All negative	2,184	72.80
All showing partial (±) or complete (+) reactions	674	22.46
Total	2,858	95.26
Results not agreeing	142	4.74
Complete total	3,000	100.00

We feel that this lack of agreement is due to two factors: (1) A larger percentage of specimens from treated, latent, and congenital cases. In 1937 specimens were chiefly from clinically diagnosed syphilitics or from presumably negative groups. With the expansion of the Venereal Disease Control Program, an increasing number of specimens come from known treated or suspected congenital cases. Mazzini[1] and Maltaner[5] have shown that in such groups the results of various tests may vary widely. A comparison with the 1937 study supports this idea.

	1937 %	1939 %
All tests negative	82.3	72.80
All tests doubtful	1.7	0.56
All tests positive	14.3	20.57
Results not agreeing	1.7	6.07

(2) With the expansion of the Venereal Disease Control Program, more specimens are submitted for the laboratory diagnosis of syphilis and a large percentage come from cases of chronic, undiagnosed illnesses. This results in an increase of nonspecific, or "false positive," laboratory tests.

Much has been written about the specificity of the various laboratory tests for syphilis. Evaluation studies have shown that the more common tests will give between 99 and 100 per cent negative reactions among presumably nonsyphilitics, and that they will detect approximately 80 to 90 per cent of known syphilitics (all types, latent, treated, etc.). In secondary syphilis the tests will detect almost 100 per cent of the cases. In such groups, our results have been well within the limits of sensitivity and specificity recommended by the United States Public Health Service.

TABLE III

DISTRIBUTION OF REACTIONS IN 182 SPECIMENS WHERE RESULTS WERE NOT IN COMPLETE AGREEMENT

(± and 1+ reactions called doubtful; 2+ and stronger called positive; Hinton test reported as − −, ±, or +; other tests as − −, ±, 1+, 2+, 3+, 4+)

HINTON			KAHN			KLINE			MAZZINI		
− −	±	+	− −	±	+	− −	±	+	− −	±	+
74	43	65	66	89	27	72	77	33	44	68	70
40.7	23.6	35.7	36.2	48.9	14.9	38.6	32.3	18.1	24.3	37.3	38.4

TABLE IV

DISTRIBUTION OF REACTIONS IN 3,000 SPECIMENS

	HINTON	KAHN	KLINE	MAZZINI
Negative	2,258	2,250	2,256	2,228
Doubtful or weakly positive	369	415	403	394
Positive	373	335	341	378
Total	3,000	3,000	3,000	3,000

Many diseases other than syphilis have been credited with the responsibility of nonspecific serologic tests for syphilis. Among these are yaws, tuberculosis, malaria, leprosy, infectious mononucleosis and numerous other diseases (particularly high febrile conditions). Recently, Krag and Lonberg[4] have stated "affections of the respiratory passages are the most frequent cause of nonspecific reactions."

The question of nonspecific reactions is complicated by the fact that in some cases it is not possible to eliminate syphilis definitely, and that the sera of nonsyphilitics may contain "reagin" at times. Judging from our experience, nonspecific reactions occur, and chiefly among presumably nonsyphilitic individuals who are suffering from chronic, undiagnosed illnesses. At first, these were dismissed as laboratory errors, but after an extensive series of checks with other laboratories, we have come to regard them as nonspecific.

Tables V and VI give in detail some of the reactions given by the tests in known syphilitics and in presumably nonsyphilitics. These cases, where there was a lack of agreement, are the only ones included, except for the presumably "nonspecific" reactions. The diagnosis and comments are those furnished by the physicians who submitted the specimens.

In addition to the results given in the preceding tables, the Kline and Mazzini tests were employed as "screening tests" in a series of 1,042 specimens from a group of college students. The results are indicated in Table VII. Complete agreement in this series was 99.51 per cent; relative agreement was 99.80 per cent.

The Kahn and Hinton tests were applied to the ten specimens which gave some reaction to the Kline or Mazzini tests. The results are given in Table VIII.

These specimens were all rechecked within a period of from seven to fourteen days. The following sera gave negative Hinton, Kahn, Kline, and Mazzini reactions upon the second test: 4, 5, 6, 7, 9, and 10. The others gave the reactions shown in Table IX.

TABLE V

REACTIONS OF THE HINTON, KAHN, KLINE, AND MAZZINI TESTS IN SERA OF KNOWN SYPHILITICS

LAB. NO.	HINTON	KAHN	KLINE	MAZZINI	REMARKS
26346	+	2+	3+	1+	Congenital
31873	- -	4+	- -	1+	Treated
31936	+	2+	3+	1+	Treated
32087	+	1+	- -	4+	Congenital
32156	- -	±	1+	- -	Treated
32304	- -	- -	2+	- -	Congenital
32331	+	1+	- -	3+	Treated
32422	+	2+	- -	4+	Congenital
32423	+	- -	4+	4+	Congenital
32966	- -	- -	1+	2+	Treated
33222	- -	- -	1+	1+	Treated
39303	- -	- -	1+	- -	Congenital
39972	+	±	2+	4+	Treated
40210	- -	±	2+	- -	Congenital
40320	- -	- -	- -	±	Treated
40623	- -	- -	- -	4+	Treated (prostitute)
41051	+	±	3+	4+	Treated
41224	+	±	2+	4+	Treated
41437	- -	- -	2+	4+	Treated
41680	+	2+	- -	3+	Treated
41707	- -	- -	±	3+	Old case, untreated
41785	+	- -	- -	2+	Treated
42531	±	- -	- -	2+	Treated
42648	+	- -	2+	3+	Treated
43371	- -	- -	- -	1+	Treated
43865	- -	- -	- -	3+	Treated
43936	±	- -	- -	3+	Congenital
43219	+	±	- -	3+	Treated
43922	+	- -	- -	3+	Old case, untreated
44231	+	±	2+	3+	Treated
44942	+	- -	- -	2+	Treated
44664	+	- -	±	3+	Primary, untreated
45157	- -	- -	- -	2+	Primary, untreated
45344	+	1+	1+	3+	Treated
47949	- -	- -	2+	3+	Treated
49699	+	- -	2+	- -	Treated
49744	+	3+	- -	- -	Treated
50902	- -	2+	- -	- -	Treated
53526	+	3+	- -	3+	Treated
54144	±	2+	- -	3+	Congenital
54162	- -	2+	2+	2+	Congenital
54678	+	±	2+	1+	Primary, untreated
56113	- -	2+	- -	2+	Old case, untreated
59917	- -	2+	- -	- -	Congenital
69209	±	- -	4+	2+	Treated
69973	±	1+	2+	1+	Old case, untreated
70588	+	2+	2+	- -	Treated
70776	- -	- -	1+	- -	Treated
74646	+	1+	2+	1+	Congenital
74671	- -	1+	2+	1+	Congenital

At the time this article was written, further investigations had not been completed. With the exception of specimen No. 8, there is good reason to believe that the reactions by sera Nos. 1, 2, and 3 are nonspecific.

Tables III and IV show that the Kline, Kahn, and Hinton tests gave approximately the same number of negative reactions, and that the Mazzini test gave fewer negative and more positive reactions. This is to be expected, however, since the sensitivity of the Mazzini test is of the same order as the Kline

TABLE VI

NONSPECIFIC REACTIONS IN PRESUMABLY NONSYPHILITIC INDIVIDUALS
(NO HISTORY OR CLINICAL SYMPTOMS OF SYPHILIS)

LAB. NO.	HINTON	KAHN	KLINE	MAZZINI	REMARKS
26210	±	2+	2+	1+	Normal*
27579	--	--	--	2+	Normal
28835	±	--	2+	±	Influenza†
28844	+	2+	2+	--	Endocarditis
39402	--	--	--	4+	Sinusitis
40237	±	±	--	±	Sinusitis†
41069	±	--	--	2+	Normal
41555	±	3+	±	±	Undulant fever†
43008	±	±	2+	3+	Jaundice†
43773	±	±	--	2+	Normal
46520	--	+	+	2+	Chronic ear abscess
58355	+	3+	3+	3+	Tularemia†
59323	+	1+	3+	3+	Normal
60344	+	--	3+	3+	Normal
60353	+	2+	2+	2+	Normal
60362	+	1+	1+	3+	Normal†
60371	±	1+	1+	3+	Normal†
60380	+	2+	3+	4+	Normal†
60389	+	1+	4+	4+	Normal†
69173	+	--	1+	3+	Recheck (No. 60344)
69182	--	1+	--	2+	Recheck (No. 60353)
69191	--	1+	2+	3+	Recheck (No. 59323)
71359	+	2+	2+	2+	Normal†
71368	+	2+	2+	--	Normal†
71858	--	1+	±	±	Psoriasis†

*The term *normal* designates specimens from healthy individuals. These were submitted in connection with physical examinations of students, food handlers, civil service applicants, etc. At the time of examination there was no clinical evidence of illness. Investigations made subsequently revealed no evidence of syphilis.

†Indicates that another specimen, submitted at a later date, gave negative reactions to all tests.

TABLE VII

KLINE AND MAZZINI TESTS ON SERA FROM COLLEGE STUDENTS

	NUMBER OF SPECIMENS	PER CENT
Kline and Mazzini both negative	1,028	98.65
Kline and Mazzini both positive (complete agreement)	9	0.86
Kline and Mazzini both positive (partial agreement)	3	0.29
Kline and Mazzini not in agreement	2	0.20
Total	1,042	100.00

TABLE VIII

HINTON, KAHN, KLINE, AND MAZZINI REACTIONS ON SERA OF COLLEGE STUDENTS

SERUM	HINTON	KAHN	KLINE	MAZZINI
1	+	1+	3+	3+
2	+	--	3+	3+
3	+	2+	2+	2+
4	+	1+	1+	3+
5	+	1+	1+	4+
6	+	2+	3+	4+
7	+	1+	4+	4+
8	+	2+	4+	4+
9	+	2+	2+	2+
10	+	2+	2+	--

exclusion test. Table IV shows a good relative agreement between the Kline, Kahn, and Hinton tests.

Table V confirms the results of Mazzini[1] and Giordano[2] as to the sensitivity of the Mazzini test, and also shows, as have Mazzini[1] and Maltaner,[5] that many persons known to have syphilis may give positive reactions with one or more tests and negative reactions with others. It is interesting to note that in such cases a less sensitive test may often give a positive reaction, while a more sensitive test may be negative.

Table V also shows the advantage of the use of a system of multiple tests, as suggested by Pierce.[6] The use of such a system has been criticized by several authors on the grounds that conflicting results are of little value to the physician. After all, he is the final judge of the laboratory report and must interpret such results in the light of clinical evidence. Although Table VI shows few cases which are presumably "false positive," or nonspecific reactions, there are many instances where clinically diagnosed cases of syphilis give similar conflicting results with the various tests. From this experience, we feel that the use of a multiple test system is warranted, and that conflicting results from several tests should not necessarily be regarded as a limitation of any particular laboratory test.

It is our opinion that as the Venereal Disease Program continues to expand, there will be an increase in the type of results given in Table V, and that an appreciation of this fact will lead to a better understanding between the laboratory and the clinician.

TABLE IX

SERUM	HINTON	KAHN	KLINE	MAZZINI	WASSERMANN
1	- -	1+	2+	3+	- -
2	±	- -	1+	3+	- -
3	- -	1+	- -	2+	- -
8	+	4+	4+	4+	4+

SUMMARY

These results confirm the findings of Mazzini and Giordano as to the specificity and sensitivity of the Mazzini test.

Since the Mazzini test is more sensitive than the Kline and Kahn diagnostic tests and the Hinton test, it gave a lower incidence of negative reactions and a higher incidence of positive findings.

The Kline, Kahn, Hinton and Mazzini tests gave a relative agreement of approximately 95.26 per cent, and an absolute agreement of 93.93 per cent. In an additional series of 1,042 specimens from college students, the Kline and Mazzini tests gave a relative agreement of 99.8 per cent and a complete agreement of 99.51 per cent.

The occurrence of nonspecific ("false positive") reactions is mentioned.

The occurrence of conflicting results of various tests in clinically diagnosed cases of syphilis is discussed.

The results, in our opinion, justify the use of a multiple test system.

In our opinion the Mazzini test is an excellent test for the laboratory diagnosis of syphilis.

We gratefully acknowledge the assistance of Mr. L. Y. Mazzini, who supplied the antigen for the Mazzini test, and the physicians who assisted us by giving case histories.

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BACTERIOLOGIC DIAGNOSIS IN GONORRHEA OF THE MALE*

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A BACTERIOLOGIC study of 221 patients with genitourinary infections is presented, and a comparison is made between the efficiency of the smear method and the cultural method as a means of diagnosis. These patients were repeatedly examined over a period of eight months. A total of 819 bacteriologic examinations, each examination consisting of smear and culture, were made on the 221 patients, of whom 160 were definitely proved to have gonorrhea by bacteriologic examination as well as by clinical evidence. Sixty-one patients were assumed to have some genitourinary infection other than gonorrhea and, while the actual cause of the infection was not established, at no time were gonococci found either by smear or culture.

METHODS

Smears: Material for smears consisted of urethral discharge, sediment of urine, and prostatic secretion, obtained after massage. The material was collected on a swab or loop and distributed with rolling or streaking motions over the glass slide.

Staining procedure: Hucker's staining method, which we modified by employing mercuric chloride as a fixative and acetone as a decolorizing agent, was used. It was found that mercuric chloride in 1 per cent solution applied for one minute to the smears would preserve the details of the cells especially well.

Cultural methods: In attempting to grow gonococcus the work of McLeod and co-workers,[1] Leahy and Carpenter,[2] and Carpenter,[3] was taken into account. The specimens for culture, which were the same as those for the smears, were collected on a sterile swab. The swab was then placed into 1.5 c.c. of broth, and the material on the swab was suspended in the broth. For technical reasons it

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was not possible to plate out the material at once, but the broth suspensions were exposed to room temperature for one and one-half to two hours before being cultured. Only in a very few cases was the glans penis cleaned, since in most cases there was not sufficient time to do it. By proper manipulations a large number of contaminations could be avoided, so that the contaminants growing on the plates would not interfere with the growth of the gonococcus. Cultures were taken only after the patients had stopped treatment for four to five days.

A 5 per cent rabbit blood chocolate agar was used, with hormone broth as a base. The blood was added to the agar, kept at a temperature of between 70° and 85° C. The final agar concentration in the medium was 1.7 per cent. The chocolate agar plates were kept in the icebox. They were not used when older than three days, since it was found that only a high amount of moisture would guarantee satisfactory growth.

Some of the broth suspension was placed on the medium by means of the swab and was then streaked out with the loop. Incubation was effected at 36° C. for about forty hours under 10 per cent carbon dioxide tension.

After forty hours' incubation the plates were inspected, and colorless, opaque colonies with irregular edges were touched with a drop of a 1 per cent watery solution of para-amino-dimethyl-aniline-monohydrochloride (Eastman Kodak), prepared fresh as soon as the solution showed some precipitate. This solution was kept in the refrigerator. If no suspicious colonies were found by mere inspection, the entire plate was flooded with the solution for a moment, and then the excess solution was poured off. Gonococcus colonies gave a typical oxidase reaction; the colonies turned pink in about ten seconds, and in the course of some minutes gradually became black. Gram-stained preparations of colonies giving a typical oxidase reaction were invariably found to consist of gram-negative diplococci. The oxidase reaction does not interfere with the characteristic staining properties of the gonococcus.

Besides gonococcus, other organisms will give the oxidase reaction. All or most of the members of the Neisseria group will give this reaction. With the exception of the meningococcus, whose colony cannot be very well differentiated from the gonococcus colony on a chocolate agar medium, and which also gives an oxidase reaction very similar to that of the gonococcus, the other members of the Neisseria group can be easily recognized from the gonococcus by their growth characteristics; they also give an oxidase reaction that is somewhat different from that of the gonococcus.

While diphtheroids do not generally give the oxidase reaction, occasionally colonies of gram-positive rods, which we assumed to be diphtheroids, were found to change their color when the oxidase stain was applied to them. However, besides being gram-positive, they could be differentiated easily from the gonococcus, giving an absolutely atypical reaction, and turning to a chalky, grayish black after fifteen to twenty minutes. When these colonies were pushed aside with the loop, the underlying medium would generally not have taken up any of the dark color, whereas the medium under gonococcus colonies, after they gave the oxidase reaction, would also be stained black. Furthermore, if a gonococcus

colony is pushed aside, a distinctly positive oxidase reaction may be obtained by applying the oxidase stain to that place of the medium where the gonococcus colony had been before. This we found did not occur with diphtheroids which gave the atypical reaction just mentioned.

In 41 cases where colonies on the basis of their morphologic character and on their property of giving a typical oxidase reaction were thought to be gonococci, the suspected colonies were isolated in pure culture and fermentation tests on an ascitic fluid agar sugar medium, as well as sodium hydroxide solubility tests, were done. In every case the tests gave the reactions characteristic of gonococcus. Besides, as a further control, all the 41 cultures were transferred to a 7 per cent horse blood agar plate and incubated at 23° to 24° C. for forty-eight hours, but without the adjustment of carbon dioxide tension. With the exception of two strains the organisms failed to grow.

On the basis of these preliminary findings in a subsequent routine procedure, all colonies, which resembled gonococcus colonies by inspection, by typical oxidase reaction, and showed gram-negative diplococci were considered to be gonococci without using fermentation tests as a means of further identification, provided these organisms were derived from the genitourinary tract of a male with a typical history indicating gonorrheal infection.

We are aware of the possibility that this method might fail to differentiate meningococci from gonococci; but in view of the rarity of meningococcal infection of the male genitourinary tract, we thought it justifiable to give out a report as positive, without going through the very time-consuming procedure of first growing the organisms in pure culture and then running fermentation tests, providing our previously stated criteria of gonococcus were satisfied.

METHOD OF REPORTING

Cultures: A culture was reported as "+" when gonococcus colonies were found, and as "0" when no such colonies were found.

Smears: A smear was reported as "+" when intracellular gram-negative diplococci resembling gonococci were found; as "±" when gram-negative diplococci resembling gonococci were found extracellularly; as "0" when such organisms were found neither intracellularly nor extracellularly.

RESULTS

A total of 819 examinations, employing both culture and smear methods simultaneously, were carried out on 221 patients.

Stain and culture agreed in 722 instances, or in 88.16 per cent.

Stain + / Culture + } 137 = 16.73% Stain 0 / Culture 0 } 585 = 71.43%

Stain and culture disagreed in 97 instances, or in 11.84 per cent.

Stain 0 / Culture + } 30 = 3.66% Stain + / Culture 0 } 5 = 0.61%

Stain ± / Culture + } 13 = 1.59% Stain ± / Culture 0 } 49 = 5.98%

The presence of gonococcus was revealed by either smear or culture method, or both methods, in a total of 185 instances of the 819 examinations. The relative efficiency of the smear or culture method for detection of the presence of gonococcus may be calculated from the following figures, taking the number 185 as 100 per cent deficiency:

Stain + / Culture +	137 = 74.05%	Stain 0 / Culture +	30 = 16.22%	Difference 13.52%
Stain ± / Culture +	13 = 7.03%	Stain + / Culture 0	5 = 2.70%	

Thus, we see that there is a decided superiority of 13.52 per cent of the cultural method over the smear method. In other words, in 13.52 per cent the culture method detected gonococci, when the smear method failed to do so. In addition, in 7.03 per cent the culture method definitely revealed gonococci when the smear method was doubtful.

Three of the 5 $\frac{\text{stain}}{\text{culture}}\ \frac{+}{0}$ findings occurred during the first two months of our investigation, when we were not as familiar with the growth conditions of gonococcus as we were later; and it is believed that in these instances the failure of the gonococcus to grow might have been due, perhaps, to too long storage of the medium with a subsequent loss of moisture.

As we have already stated, the report "±" referring to the smear signified "gram-negative diplococci, resembling gonococci, situated extracellularly." Thus, in reporting a smear as "±," it was necessary in the first place to decide whether the extracellular organisms were gram-negative, then whether they were diplococci, and finally whether they resembled the gonococcus. While it is often an easy matter to arrive at the report "±," there is a considerable number of smears where the decision is difficult, and the personal equation enters as in no other phase of the work. In many smears where the culture exhibited staphylococci and diphtheroids only, the smear showed besides gram-positive cocci, cocci that were not definitely gram-positive or gram-negative, associated at times in diplococcus formation. The decision whether these organisms are also staphylococci, which just did not stain as well as the others, or whether they should be regarded as being possibly gonococci, is entirely a matter of personal judgment, and even the same observer looking at a given smear today may come to a different decision tomorrow.

Taking the total of 819 smears and cultures as 100 per cent, we see that in 62 instances (7.57 per cent) the smear was reported as "±." In 13 of these instances (1.59 per cent) the diagnosis was settled by the simultaneous positive culture. In 49 instances (5.98 per cent) the culture proved to be negative. These 49 doubtful "±" smears occurred on 43 patients, 11 of whom were regarded as being affected with some genitourinary infection other than gonorrhea, and on whom at no time a definite positive finding had been obtained. The other 32 patients had clinical gonorrhea confirmed on admission by smear and culture, but who at a later date exhibited a "±" smear along with a negative culture.

It was at first difficult for us to correlate the "±" findings of the smears with the simultaneous negative findings of the cultures, since it may have been possible that the suspicious organisms found in the smear were gonococci, which just would not grow under the conditions to which they were subjected by our methods. However, when more data were available, it was felt that in judging whether the organisms responsible for the "±" report of a smear were gonococci or not, the deciding factor should be the culture, in the sense that if no gonococci were found on the culture, the suspected organisms in the smear should not be assumed to be gonococci. This assumption seemed to be justified by the following reasoning: It was very unlikely that in the case of a doubtful "±" smear and a negative culture we would deal with a strain of gonococcus that would not grow under our methods, since a positive culture was obtained in every case where these patients showed a definitely positive smear on admission.

We further undertook a follow-up study of the patients who exhibited a $\frac{\text{stain}\ \pm}{\text{culture}\ 0}$ report, with the idea that if the suspicious organisms in the "±" smear were gonococci, the symptoms of the patients might again exacerbate and culture and stain become positive. The findings of this study are as follows:

In the large majority of patients there were several reports of negative smears and cultures preceding the "±" smear. Five patients were found unsatisfactory for any analysis, either because they did not return or because they allowed themselves to be exposed during the time of observation. Twenty-six patients, of whom 12 have been definitely dismissed as cured, showed only $\frac{\text{stain}\ 0}{\text{culture}\ 0}$ findings after the $\frac{\text{stain}\ \pm}{\text{culture}\ 0}$ report. Fourteen of the 26 patients have not been dismissed at this time, and show repeatedly a $\frac{\text{stain}\ 0}{\text{culture}\ 0}$ report after the $\frac{\text{stain}\ \pm}{\text{culture}\ 0}$ report. Most of these have been put through provocative measures as massage, introduction of sound, permission of alcohol and coitus, without either culture or stain becoming positive. Three of the 14 patients not yet dismissed had, we believe, reinfections. For a period of two and one-half to three months after the $\frac{\text{stain}\ \pm}{\text{culture}\ 0}$ report, during which these patients were examined repeatedly, no positive or "±" findings occurred. These patients were finally permitted to stay away for twenty-eight days; they returned with an acute discharge. One of the patients admitted exposure without prophylaxis.

Only in one of the 32 patients was there a $\frac{\text{stain}\ 0}{\text{culture}\ +}$ finding after the $\frac{\text{stain}\ \pm}{\text{culture}\ 0}$ report.

We admit that this method of analyzing the significance of a "±" finding on the smear by follow-up studies is very much open to criticism. However, we thought that some additional information might thus be gained.

Reviewing this analysis, we see that in only 5 instances of 819 bacteriologic examinations, an incidence of 0.61 per cent, where the stain was positive and the simultaneous culture was negative, did the stain detect something important that the culture did not reveal.

CONCLUSIONS

1. A total of 819 bacteriologic examinations, employing simultaneously culture and smear methods as a means of diagnosis, were carried out on 221 patients.

2. The culture and smear methods agreed in 88.16 per cent and disagreed in 11.84 per cent of the cases, taking the 819 examinations as 100 per cent.

3. In 185 instances the presence of gonococci was revealed by either smear or culture, or both methods. Taking the number 185 as 100 per cent efficiency in detecting gonococci, we find that the culture was able to detect gonococci in 13.52 per cent, when the smear definitely failed to do so. In addition, in 7.03 per cent, the culture definitely revealed gonococci, when the smear was doubtful.

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EXPERIMENTAL THROMBOCYTOPENIC PURPURA IN THE GUINEA PIG*

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SINCE Werlhof first gave an accurate clinical description of essential thrombocytopenic purpura, it has been known that a marked reduction of the circulating blood platelets is a characteristic feature of the condition. However, many students of this disease believe that other factors must be in operation to produce the clinical picture, and it has been assumed that some change in the capillaries must be the ultimate cause of the leakage of blood through them.

This study was planned as an attempt to estimate the relative roles played by the blood platelets and the capillaries. Our approach to the problem was as follows: (1) by producing thrombocytopenia, and (2) by producing increased capillary fragility.

Experimental Production of Thrombocytopenia.—Numerous investigators have studied the effects of various substances upon blood platelets.[2-21]

Duke[2] was apparently the first in this country to attempt to produce thrombocytopenic purpura, and for this purpose he used benzol. Ledingham[3]

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in 1914 reported the results of an experiment in which thrombocytopenic purpura was produced in guinea pigs following injections of an antiplatelet serum which was capable of selectively destroying circulating platelets. Ledingham and Bedson,[4] and Bedson,[15, 19, 20] after studying the properties of this serum, concluded that it contained a specific antibody capable of lyzing and agglutinating blood platelets in vivo, without affecting the other formed elements of the blood. Tocantins[7, 8] studied the thrombocytopenic purpura produced in dogs with an antiplatelet serum, and more recently Troland and Lee[21] produced thrombocytopenic purpura in rabbits with an extract obtained from the spleen of patients with thrombocytopenic purpura.

Experimental Production of Increased Capillary Fragility.—Our problem was to find a method for producing capillary damage that could be maintained over long periods, that would in itself not cause purpura, and that could be controlled to some extent. We employed vitamin C deficiency as the method that best fulfilled these conditions. Our attempt was to produce vitamin C depletion without reaching the point of definite clinical scurvy. Vitamin C has been regarded by many as an important factor in the hemorrhagic diseases, from both the etiologic and therapeutic standpoints.[22-32] In recent years capillary fragility has been employed extensively as an indirect measure of the degree of vitamin C storage.[33-41]

METHODS

An antiplatelet serum was prepared in the following manner: One hundred to 130 c.c. of blood were obtained from guinea pigs by cardiac puncture. The blood was collected in an equal volume of 3.8 per cent sodium citrate in normal salt solution. The platelets were extracted from the solution by fractional centrifugalization, and after being suspended in 10 c.c. physiologic salt-solution, were injected intravenously into a rabbit. This procedure was carried out at intervals of five to seven days, until the rabbit had received from three to six injections. At the end of this time the blood was withdrawn from the rabbit by cardiac puncture, stored in the refrigerator overnight, and the serum separated out and collected in sterile bottles. Because it was found that the antiplatelet serum lost potency in a relatively short time, all subsequent sera were lyophilized according to the technique of Flosdorff and Mudd.[42]

Hemoglobin determinations were made with standardized Sahli instruments in which 100 per cent equal 13.8 Gm. of hemoglobin per 100 c.c. of blood. Erythrocyte and leucocyte counts were made with standard pipettes and a Neubauer counting chamber, all instruments being standardized by the United States Bureau of Standards. Platelets were counted directly in the counting chamber after diluting 1:100 in the standard red blood cell pipettes with Reese and Ecker fluid.[1] A period of ten minutes was allowed to elapse from the time the fluid was placed in the counting chamber until the count was begun.

Capillary resistance was measured by the Dalldorf technique,[37] employing the da Silva-Mello instrument. We used the skin over the lower portion of the abdomen, which had been carefully shaved at least twenty-four hours before the readings were taken.

Twelve young guinea pigs, weighing between 200 and 300 Gm., were chosen. Six were segregated and placed upon a diet consisting of baked skimmed milk, 30.0 per cent; butter fat, 10.0 per cent; rolled oats and bran, 56.0 per cent; sodium chloride, 0.5 per cent; Osborne and Mendel salt mixture, 1.5 per cent; dried yeast, 1.0 per cent; and cod-liver oil, 1.0 per cent. This group was designated as the scurvy (S) group.

Six animals were placed upon a diet consisting of barley, alfalfa, salt, and greens (lettuce and cabbage) supplemented with 2 c.c. of orange juice daily. This was designated as the control (C) group.

TABLE I

EFFECT OF DIET ON WEIGHT, BLOOD, AND CAPILLARY FRAGILITY OF GUINEA PIGS

TWO GROUPS OF SIX GUINEA PIGS EACH	ANIMALS ON ADEQUATE VITAMIN C DIET			ANIMALS ON VITAMIN C DEFICIENT DIET		
	NUMBER OF DETERMINATIONS	STANDARD DEVIATION ± P.E.	MEAN ± P.E.	NUMBER OF DETERMINATIONS	STANDARD DEVIATION ± P.E.	MEAN ± P.E.
Weight in grams	40	114 ± 8.65	371 ± 77	26	57 ± 5.3	316 ± 38.3
Hemoglobin percentage	34	4.36 ± 0.38	74 ± 0.49	36	5.93 ± 0.47	73 ± 0.68
Erythrocytes	32	310,000 ± 26,400	5,640,000 ± 35,000	37	500,000 ± 39,400	5,370,000 ± 56,000
Leucocytes	33	2130 ± 178	9100 ± 240	36	4650 ± 372	9600 ± 530
Platelets	47	101,500 ± 7,100	473,000 ± 9,736	47	178,600 ± 12,500	460,000 ± 17,693
Capillary fragility	16	0 - 5 at 15 cm.		16	3 - 35+ at 15 cm.	

EXPERIMENTAL

Effect of Diet on the Weight, Blood Picture, and Capillary Fragility of Guinea Pigs.—The results are shown in Table I. There was a definite influence of vitamin C deficiency on growth. These animals were at the age when rapid growth is expected, and, although the vitamin C deficiency animals showed some increase in weight, it was neither as uniform nor as great as that shown by the animals receiving vitamin C.

The values for hemoglobin, erythrocytes, leucocytes, and blood platelets were statistically similar in the two groups, whereas the capillary fragility showed considerable difference. The vitamin C deficiency animals showed a distinctly greater capillary fragility when measured by the Dalldorf method. The effect of the deficiency was more marked on the capillaries than on the formed elements of the blood.

Effect of Antiplatelet Serum on Guinea Pigs With Vitamin C Deficiency and With High Vitamin C Diets.—Six guinea pigs, three with vitamin C deficiency and high capillary fragility and three on diets high in vitamin C and with low capillary fragility, were chosen. The animals were given antiplatelet serum intraperitoneally. Four received 0.2 c.c. and two of the control group received 0.3 c.c. The results (Table II and Fig. 1) were strikingly similar. Before the injection of antiplatelet serum, the average platelet count was 600,000 per cubic millimeter in the vitamin C deficient animals and 710,000 per cubic millimeter in the control animals. Twenty-four hours after the injection the platelet count had fallen to 70,000 and 40,000 per cubic millimeter, respectively. This was

associated with the appearance of purpura, increased bleeding time, and poor clot retraction. The clot was so soft and friable that twenty-four hours after the previous skin puncture, the clot could be wiped away and persistent oozing would follow. Forty-eight hours after the injection, the platelets began to increase again, reaching by the fifth day 380,000 per cubic millimeter in the vitamin C deficient animals and 400,000 per cubic millimeter in the controls. A second injection of the serum (0.2 c.c.) again produced a rapid drop in platelets; this was followed by the appearance of purpura. The platelet drop was very marked within six hours after the injection, but was maximal at twenty-four hours.

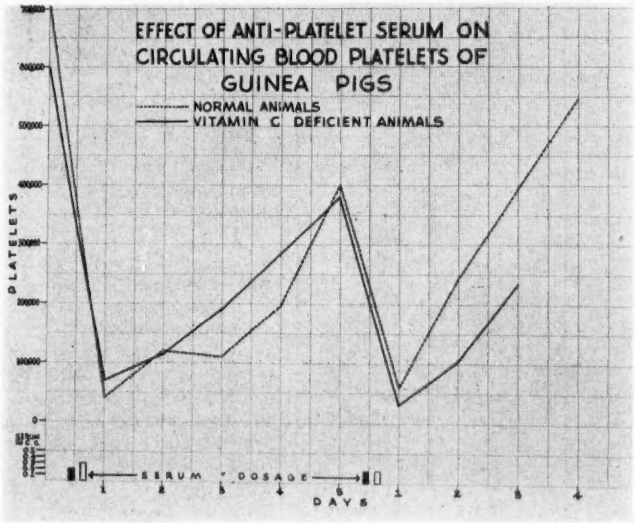

Fig. 1.

Two of the animals (control) died, one forty-eight hours and one fifty-two hours after the injection. Necropsy showed bleeding into the internal organs and serous membranes. Death was apparently due to exsanguination. In all surviving animals the purpuric lesions disappeared in from three to five days.

Effect of Normal Rabbit Serum on Guinea Pigs Deficient in Vitamin C.—In order to determine whether normal rabbit serum had any effect upon blood platelets, injections ranging in dosage from 0.2 to 1.0 c.c. were given to five animals with vitamin C deficiency. The data are shown in Table III. There was no significant variation in the platelet levels and no evidence of a bleeding tendency. This definitely indicates that (1) the platelet-destroying property of the antiserum is not inherent in the rabbit serum; (2) normal rabbit serum will not alter the capillary permeability sufficiently to cause bleeding.

Effect of Increasing Doses of Antiplatelet and Normal Rabbit Serum on Vitamin C Deficient Guinea Pigs.—Two vitamin C deficient animals were given injections of antiplatelet serum in increasing doses, ranging from 0.05 c.c. to 0.3 c.c. One animal was given normal rabbit serum in the same manner. (Results are shown in Tables IV and V and Fig. 2.) The smallest dose (0.05 c.c.)

TABLE II

EFFECT OF ANTIPLATELET SERUM ON VITAMIN C DEFICIENT GUINEA PIGS

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
S-11		Before	530,000	
	0.2	24 hr.	68,000	Entire body covered with petechiae and purpuric spots. Bleeding time prolonged
		48 hr.	126,000	No fresh petechiae
		3 days	182,000	Petechiae fading
		4 days		Petechiae gone. Ecchymoses fading
		5 days	364,000	
	0.2	24 hr.	32,000	Entire skin covered with fresh petechiae
		48 hr.	80,000	Petechiae starting to fade
		3 days	302,000	Petechiae gone
S-12		Before	692,000	
	0.2	24 hr.	62,000	Entire body covered with purpuric spots. One on back 3 cm. in diameter
		3 days	175,000	Hemorrhagic spots fading
		4 days		Ecchymoses fading but still visible
		5 days	278,000	Spots faded
	0.2	24 hr.	44,000	Entire body covered with fresh spots and pin-point petechiae. Bleeding time prolonged
		48 hr.	142,000	Petechiae fading. Bleeding time not prolonged
		3 days	282,000	Petechiae gone. Ecchymoses still visible
S-13		Before	594,000	
	0.2	24 hr.	86,000	Body covered with purpuric spots and fine petechiae. Bleeding prolonged. Animal lively
		48 hr.	104,000	No fresh petechiae
		3 days	216,000	Hemorrhagic spots fading
		4 days		Larger ecchymoses still visible
		5 days	498,000	Hemorrhagic spots gone
	0.2	24 hr.	12,000	Bleeding prolonged. Pin-point petechiae and small bright red ecchymotic spots over entire body
		48 hr.	92,000	No new spots
		3 days	224,000	Petechiae almost entirely faded

EFFECT OF ANTIPLATELET SERUM ON GUINEA PIGS ON HIGH VITAMIN C INTAKE

C-11		Before	550,000	
	0.3	6 hr.	48,000	Ear bled for one hour after puncture
		24 hr.	40,000	Blood still oozing from ear
		48 hr.		Animal dead. Organs exsanguinated. Ecchymoses into pericardium, lungs, skin, renal cortices, and wall of stomach and bowel. Stomach filled with blood clot
C-12		Before	760,000	
	0.3	6 hr.	96,000	No bleeding
		24 hr.	50,000	Skin covered with petechiae and ecchymoses
		2 days	120,000	No fresh purpura
		3 days	110,000	Petechiae fading. Ecchymoses still visible
		4 days	186,000	Purpura fading
		6 days	408,000	Ecchymoses gone
	0.2	7 hr.	72,000	Ear bled excessively from puncture
		24 hr.	60,000	Skin covered with fresh petechiae
		48 hr.	244,000	Petechiae fading
		4 days	548,000	Petechiae gone
C-13		Before	800,000	
	0.2	6 hr.	64,000	Ear bled profusely from puncture
		24 hr.	56,000	Oozing at site of former puncture. Skin covered with petechiae and large ecchymotic areas
		48 hr.		Moribund
		50 hr.		Dead. Bled into skin, serous membranes, lungs, pancreas, liver, stomach, and colon

TABLE III

EFFECT OF NORMAL RABBIT SERUM ON GUINEA PIGS WITH VITAMIN C DEFICIENCY

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
N-1	0.2	Before	580,000	No evidence of bleeding
		6 hr.	496,000	
		24 hr.	428,000	
	0.4	6 hr.	400,000	
		24 hr.	365,000	
N-2	0.2	Before	585,000	No evidence of bleeding
		6 hr.	646,000	
		24 hr.	542,000	
	0.4	6 hr.	570,000	
		24 hr.	520,000	
N-3	0.2	Before	620,000	No evidence of bleeding
		6 hr.	612,000	
		24 hr.	634,000	
	0.4	6 hr.	510,000	
		24 hr.	570,000	
N-4	0.8	Before	408,000	No evidence of bleeding
		6 hr.	400,000	
		24 hr.	480,000	
N-5	1.0	Before	342,000	No evidence of bleeding
		6 hr.	326,000	
		24 hr.	348,000	

TABLE IV

EFFECT OF INCREASED AMOUNTS OF ANTIPLATELET SERUM ON GUINEA PIGS WITH VITAMIN C DEFICIENCY

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
S-16	0.05	Before	746,000	No evidence of bleeding
		6 hr.	700,000	
		24 hr.	254,000	
	0.1	6 hr.	252,000	
		24 hr.	100,000	
	0.2	Before	308,000	
		6 hr.	94,000	
		24 hr.	88,000	
	0.3	6 hr.	90,000	
		24 hr.	70,000	
S-17	0.05	Before	400,000	No bleeding
		6 hr.	370,000	
		24 hr.	304,000	
	0.1	6 hr.	222,000	
		24 hr.	56,000	
	0.2	6 hr.	40,000	Prolonged bleeding time. Fine petechiae scattered over entire body
		24 hr.	34,000	

of antiplatelet serum was followed by a drop in the platelet level (from 570,000 to 280,000 per cubic millimeter), but this was not regarded as being significant. Following an injection of 0.1 c.c., the platelet level dropped to 75,000 per cubic millimeter. Larger doses produced no further significant decrease in the platelet level, but a dose of 0.2 c.c. was required to produce purpura and increased bleeding time.

Following injections of serum from a normal rabbit, there was no significant change in the level of blood platelets. There was variation, ranging from 368,000 to 650,000 per cubic millimeter (Table V and Fig. 2), but this was within the probability of normal variation, as established by the statistical constants.

Apparently the maximum effect of the antiplatelet serum cannot be exceeded by increasing the dosage, but is inherent in the potency of the serum.

TABLE V

EFFECT OF INCREASED AMOUNTS OF NORMAL RABBIT SERUM ON GUINEA PIG WITH VITAMIN C DEFICIENCY

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
S-15		Before	620,000	
	0.05	6 hr.	600,000	
		24 hr.	650,000	
	0.1	6 hr.	614,000	
		24 hr.	550,000	No evidence of bleeding at any time
		Before	368,000	
	0.2	6 hr.	400,000	
		24 hr.	450,000	
	0.3	6 hr.	400,000	
		24 hr.	400,000	

TABLE VI

EFFECT OF ANTIPLATELET SERUM DILUTED WITH NORMAL RABBIT SERUM ON PLATELETS OF NORMAL GUINEA PIGS

ANIMAL	AMOUNT IN C.C.		INTERVAL AFTER INJECTION	PLATELETS	REMARKS
	ANTI-PLATELET SERUM	NORMAL RABBIT SERUM			
P-1			Before	300,000	
	0.1 +	0.9	24 hr.	286,000	No bleeding
	0.3 +	0.7	24 hr.	32,000	Skin covered with petechiae Bleeding time prolonged
P-2			Before	250,000	
	0.2 +	0.8	24 hr.	60,000	A few scattered petechiae behind the ears
	0.4 +	0.6	24 hr.	28,000	Numerous petechiae and several ecchymoses
P-3			Before	480,000	
	0.5 +	0.5	24 hr.	44,000	Skin and mucous membranes covered with petechiae and ecchymoses Bleeding time prolonged
			48 hr.	Dead	Peritoneal cavity filled with free blood The serous membranes were covered with ecchymoses
P-4			Before	536,000	
	0.7 +	0.3	24 hr.	24,000	Skin covered with petechiae and ecchymoses Bleeding time prolonged

Effect of Antiplatelet Serum Diluted With Normal Rabbit Serum.—We attempted to find whether dilution would result in loss of potency and whether the admixture of normal serum would result in changes in the antiplatelet properties of the antiplatelet serum. Four normal guinea pigs were given injections

of mixtures of antiplatelet serum and normal rabbit serum. The total volume of serum injected was 1 c.c., of which the antiplatelet portion ranged from 0.1 to 0.7 c.c. There was no difference between the response to the mixed serum and that which had been observed using the straight antiplatelet serum (Table VI). When the proportion of antiplatelet serum reached 0.2 c.c., there followed within twenty-four hours a marked platelet drop (from 250,000 to 60,000 per cubic millimeter) associated with hemorrhagic phenomena.

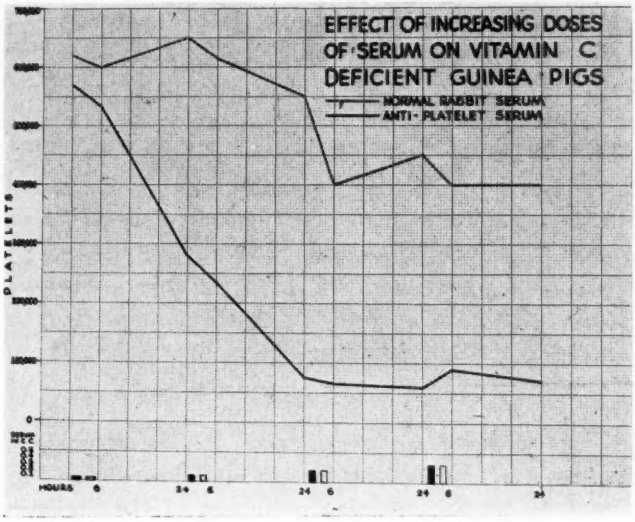

Fig. 2.

DISCUSSION

It is difficult to draw definite conclusions when dealing with experiments that present uncontrollable variables. However, we feel that certain facts appeared consistently enough to warrant attention. The most striking and consistent factor was the apparent relationship between the blood platelet level and the bleeding time. Generally, when the platelet level fell below 70,000 per cubic millimeter, a condition was produced similar to that commonly seen in human thrombocytopenic purpura, i.e., prolonged bleeding time, normal clotting time, poor clot retraction, and bleeding into the skin, mucous membranes, and internal organs. This was not always the case, as instances were observed when a low platelet count was not associated with purpuric manifestations. The rapidity of the fall in the platelet level, as well as the absolute number of circulating platelets, appeared to be important factors. This may explain why very small doses of serum, although capable of producing thrombopenia, were unable to cause bleeding.

This study was predicated upon the assumption that a capillary lesion, in addition to a platelet deficiency, is necessary for purpura to occur. The presence of a capillary lesion was assumed when an increased capillary fragility was shown by the Dalldorf technique for estimating capillary fragility. In evaluating the part played by the capillary lesion in thrombocytopenic purpura, we considered that the capillary bed in vitamin C deficiency might be very per-

meable and allow capillary hemorrhages to occur, with slight reductions in the platelet level, i.e., a level above that necessary to cause purpura in a normal animal. As has been found in these experiments, such a premise was not warranted. Unfortunately, we have no means of showing mathematical correlation between vitamin C deficiency, capillary fragility, and purpura, but so far as our experiments are concerned, there appears to be none.

This does not mean that vitamin C is not concerned with capillary fragility, but it does indicate that the type of capillary change which we assume to be present in thrombocytopenic purpura is of a different order. Treatment of this disorder with vitamin C does not appear to have a rational basis.

Our findings fail to verify our original conception that an anatomic capillary lesion must be present before hemorrhage can occur. It is more likely that the passage of blood through the walls of the vessels is determined by the functional state of the capillaries, i.e., their degree of dilatation. This vasodilatation, when associated with a considerable platelet deficiency, may be the mechanism by which hemorrhage is produced. Capillary dilatation may be initiated by the rapid disintegration of platelets liberating some substances possibly of a histamine-like nature. We have no proof of this hypothesis, but experiments are under way to investigate the mechanism of capillary dilatation.

SUMMARY

An antiplatelet serum has been produced that is capable of selectively destroying blood platelets and causing purpura. The production of purpura is apparently related to the quantitative reduction in the number of circulating platelets and to the rapidity of the fall in the platelet level.

The effect of the serum on animals with anatomically intact capillaries and on animals with capillary lesions (vitamin C deficiency) is the same. This suggests that the capillary factor is functional rather than anatomic and could be more profitably studied by physiologic means. The hypothesis is advanced that capillary dilatation in addition to a platelet deficiency is necessary before hemorrhage can occur.

Vitamin C does not protect guinea pigs against experimentally produced thrombocytopenic purpura.

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THE DEPRESSOR EFFECT OF POTASSIUM SULFOCYANATE BEFORE AND AFTER BILATERAL SPLANCHNICOTOMY IN NORMAL AND HYPERTENSIVE DOGS*

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IN ANOTHER paper we[1] have reported our experimental and clinical experiences with the administration of potassium sulfocyanate to patients with essential hypertension and to dogs with hypertension produced by the Goldblatt ischemic method.

It has been shown that a certain number of patients with essential hypertension react favorably to the administration of potassium sulfocyanate if the individual dosage is carefully controlled by blood cyanate level determinations. Accompanying the fall in the systolic and diastolic blood pressures, there is a decrease in the blood serum proteins; the blood cholesterol falls; the sedimentation rate increases markedly and the hematocrit reading decreases. These favorable reactions to the cyanates in patients have been confirmed in the experimental laboratory by administering the drug to normal and hypertensive dogs.

In another group of patients with essential hypertension, the response to the cyanates may be poor, and attempts to obtain a more favorable effect by increasing the level of the cyanates in the blood are followed by symptoms of cyanate toxicity which require that the administration of the drug be stopped.

We have also reported that after removal of the splanchnic nerves supradiaphragmatically in those patients with essential hypertension who have responded poorly to cyanate therapy, a certain number definitely react more favorably to the drug. The blood pressure levels, both systolic and diastolic, the blood cholesterol, serum proteins, hematocrit, and sedimentation rate accurately reflect this conversion to a satisfactory response.

As yet no one has been able to explain satisfactorily the effect of potassium sulfocyanate in the treatment of essential hypertension. Claude Bernard and other earlier investigators believed that sulfocyanate exerted its effect because it was a direct muscle poison and abolished muscular activity, a theory long since disproved. Westphal and Blum[2] suggested on purely theoretical grounds that cholesterol, as well as protein cleavage products, decreased the permeability of the cell membrane of the smooth muscles of the arterioles. Such a condition, they believed, would favor an excessive retention of pressor substances in the cell normally present for the maintenance of tonus, and thus persistent arterial hypertonus would result. They further stated that the salt action of the cyanate ion might act upon the cholesterol at the cell membrane to produce an increase

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in the permeability of the arterial muscle cells, thus allowing an escape of the retained pressor substance which would result in a lowering of arterial tension. Although this explanation has never been accepted fully, some support is afforded by Gellhorn[3] who observed that weak solutions of cyanate ion permeated the isolated striated muscle of a frog so slowly that no contraction occurred, but that when that same muscle containing cyanate ion was transferred to a cyanate-free solution, withdrawal of the ion was so rapid that actual contracture was produced. Thus he concluded that the ion had definitely increased the permeability of the muscle cell membrane.

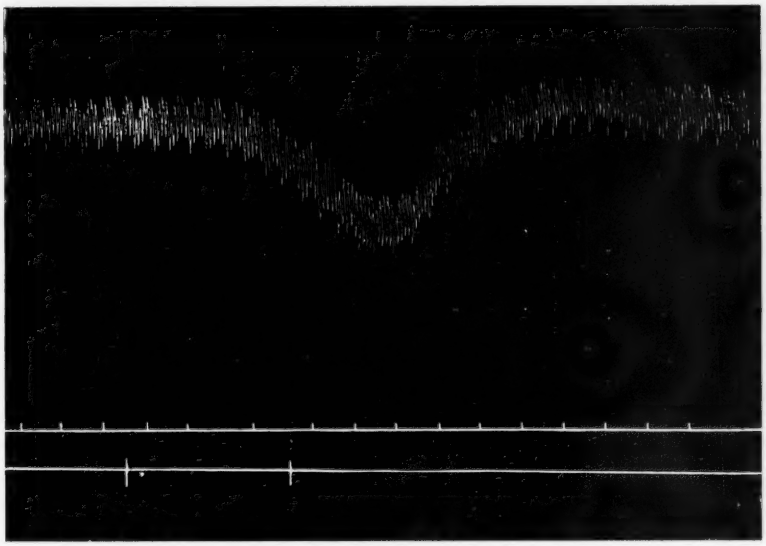


Fig. 1.—Blood pressure response of a normal dog (weight, 17.2 kg.) to an intravenous injection of 0.25 Gm. of potassium sulfocyanate.

In an effort to learn something of the method of action of potassium sulfocyanate, a series of acute experiments were carried out upon six dogs with experimental ischemic hypertension, twelve normal dogs, and four normal dogs to which potassium sulfocyanate had been administered over a sufficiently long period of time to establish a high blood cyanate level. In all of the hypertensive animals, in seven of the normal dogs, and in all of the normal dogs with blood cyanate levels, blood pressure tracings were taken before and after bilateral removal of 3 to 4 inches of the thoracic sympathetic trunks and ganglia above the diaphragm and section of the greater and lesser splanchnic nerves. Blood pressure tracings were made with a cannula in the carotid artery, and injections of potassium sulfocyanate were made into the femoral vein. A tracheal cannula connected with an oxygen tank and an automatic respirator was used in each experiment and successfully combated the respiratory difficulties which attend opening the thoracic cavity bilaterally. In some of the early experiments a record of the outflow from the coronary artery was made.

The results of these acute experiments can be stated briefly because they were uniform when the dosage did not exceed the tolerance for the particular

dog under observation and when the reaction of the dog to the potassium ion in the drug did not produce a rise in the blood pressure and cardiac fibrillation which resulted in death. We were never able to predict which animal would exhibit these reactions. If the animal's tolerance was exceeded with the initial injection, death occurred promptly. That cardiac fibrillation was the reaction of the heart to the potassium ion in the drug, with a resulting sharp rise in the blood pressure, was proved in several animals by the injection of sodium sulfocyanate without such an effect. Occasionally, in some normal dogs the pressor effect of the potassium ion overshadowed the common depressor effect of the cyanates.

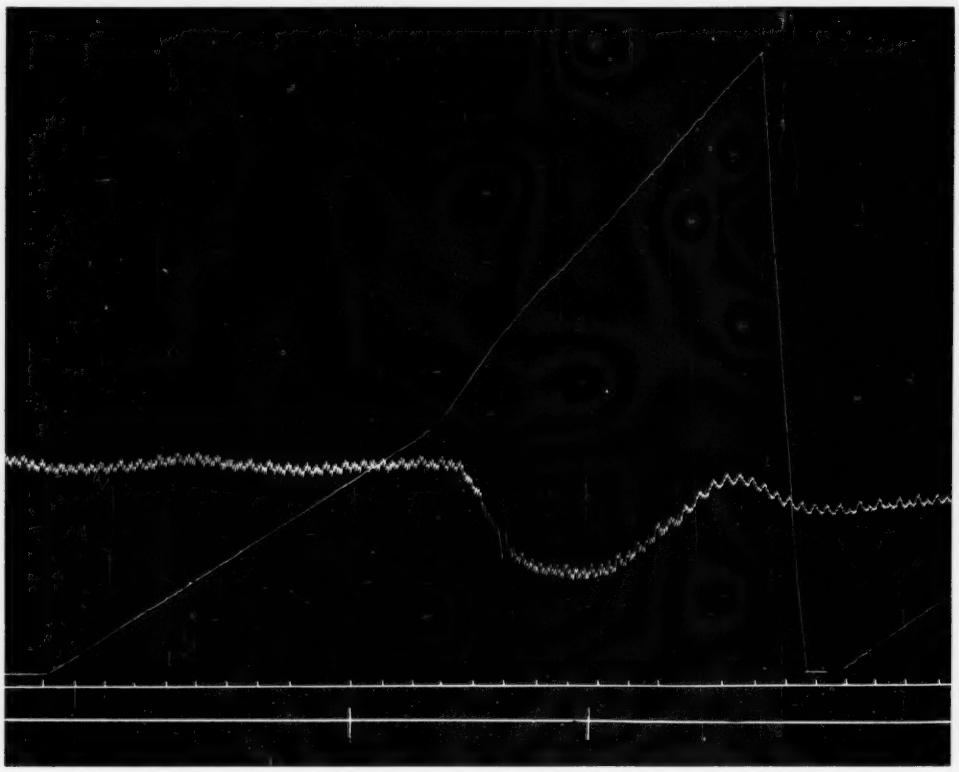
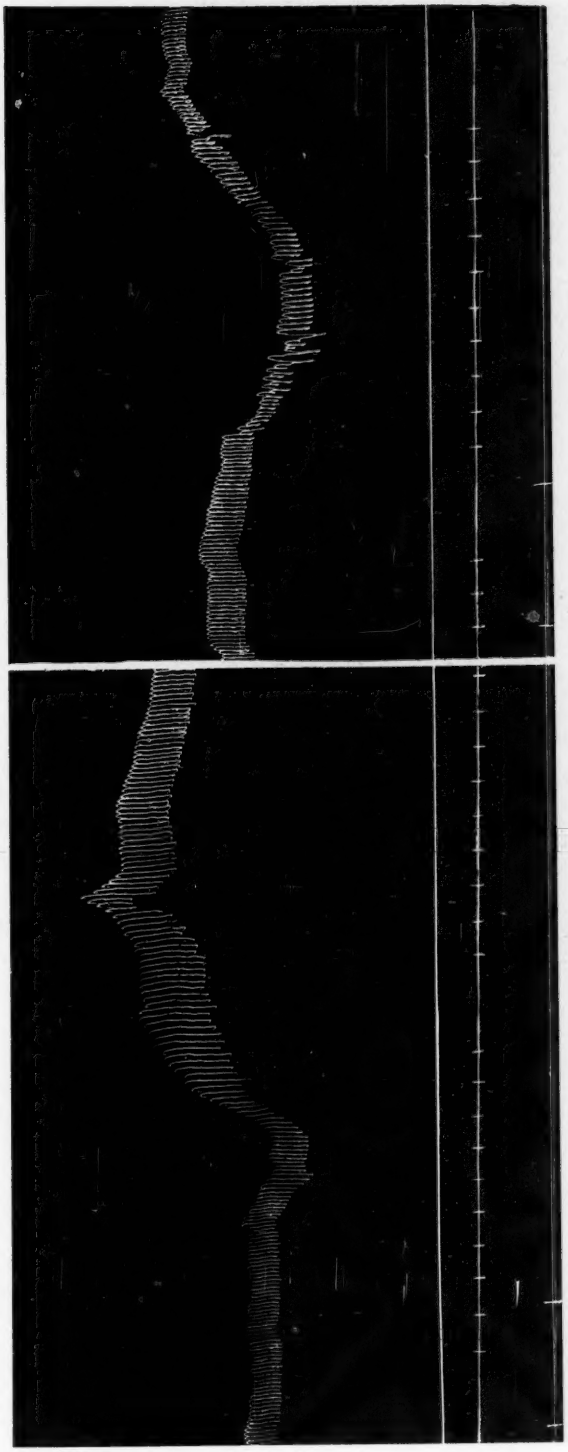


Fig. 2.—Blood pressure response and coronary outflow of normal dog (weight, 19 kg.) to intravenous injection of 0.50 Gm. of potassium sulfocyanate. Note sudden increase in coronary outflow, coincident with fall in pressure.

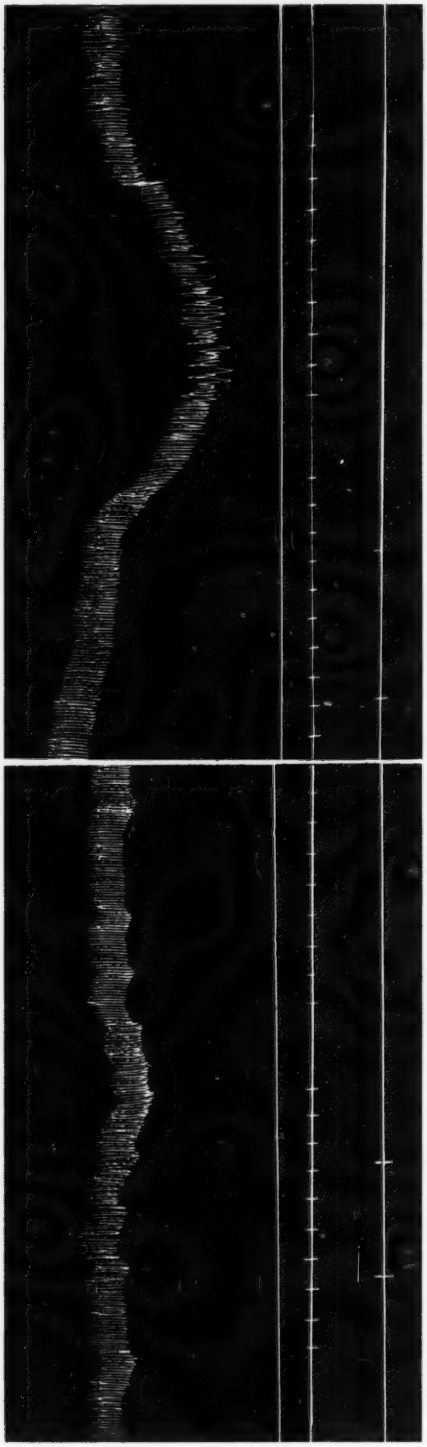
The usual prompt fall in blood pressure following injection of potassium sulfocyanate intravenously in small doses (0.013 Gm. per 1 kg.) makes it difficult to regard the effect as based upon anything other than a vasodilator mechanism (Fig. 1). The increased coronary outflow (Fig. 2) would seem to corroborate this view. Any explanation for the action of the cyanates as a protoplasmic toxic agent would not appear logical in the presence of such a prompt response. Fig. 3*A* illustrates the beginning of a blood pressure drop following an injection of potassium sulfocyanate which is quickly overtaken by the pressor action of the potassium ion and evidence of a beginning cardiac arrhythmia. Fig. 3*B* illustrates the more definite depressor response in this same animal to a similar dose of potassium sulfocyanate after the splanchnic nerves



B.

A.

Fig. 3.—Blood pressure responses in normal dog (weight 18.2 kg.) to an intravenous injection of 0.50 Gm. of potassium sulfocyanate (*A*) before and (*B*) after supradiaphragmatic splanchnicotomy.



A.

B.

Fig. 4.—Blood pressure responses in dog with experimental hypertension (weight 20.9 kg.) and a blood cyanate level of 33 mg. to an intravenous injection of 0.50 Gm. of potassium sulfocyanate (*A*) before and (*B*) after supradiaphragmatic splanchnicotomy.

were sectioned. This would seem to point definitely to the effect of the removal of the vasoconstrictor mechanism in the visceral area supplied by the splanchnics. Similar results were obtained upon dogs which had been given cyanates over a period of several days in order to establish a blood cyanate level. It was noted, however, that it required a smaller dose of the drug to produce a similar quantitative response in the blood pressure in these animals.

Fig. 4 (*A* and *B*) illustrates the response of the blood pressure in a hypertensive dog in which a partial ischemia of both kidneys had been produced several months previously by the application of Goldblatt clamps to the renal arteries. This particular animal had been fed potassium sulfocyanate for several weeks and had been proved to be responsive to the drug, as evidenced by a fall in the mean arterial pressure and significant changes in the blood chemistry. Before this experiment was carried out, cyanate administration had been stopped and the blood pressure had returned to its previous high level. Fig. 4*B* shows the increased response after splanchnicotomy to the same dose of potassium sulfocyanate as was injected in obtaining the tracing shown in Fig. 4*A*. Again the promptness of the response to injection of the cyanates speaks for a direct vasodilator action as against a general toxic depressor effect.

SUMMARY

From the results of acute experiments carried out upon normal dogs, ischemic hypertensive dogs, and normal dogs with elevated blood cyanate levels, it would seem logical to believe that the effect of the intravenous injection of potassium sulfocyanate produces its depressor effect as the result of a general vasodilatation. This effect can be increased by removal of the thoracic sympathetic trunk and splanchnic nerves supradiaphragmatically. These experimental observations lend support to the observation made clinically that in many instances patients who respond poorly to cyanate therapy may become sensitive following a bilateral splanchnicotomy.

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THE ACID-BASE BALANCE AND WATER CONCENTRATION OF THE BLOOD DURING THE TOXEMIAS OF LATE PREGNANCY*

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A NUMBER of reports on the acid-base balance in the blood during the toxemias of late pregnancy have appeared recently.[1-5] Although there is some variation, depending upon the type and severity of the toxemia, it is generally agreed that the acid-base values are within the accepted range for normal pregnant women, except shortly after a convulsive seizure, when the bicarbonate and pH values are abnormally low, and during coma, when there is an acidosis caused by uncompensated alkali deficit (Van Slyke, area 9).

For the purpose of this study, the toxemias of late pregnancy are divided into three clinical groups: (1) chronic cardiovascular renal disease (essential hypertension, arteriolar sclerosis), (2) toxemia without convulsions, and (3) toxemia with convulsions (eclampsia). This classification has been employed in the clinic for some years and has proved to be satisfactorily simple. Patients representing each of these three groups were studied to determine the water concentration of the whole blood and the acid-base balance of the plasma by methods similar to those employed in investigating the blood changes in normal women during late pregnancy.[6, 7]

METHODS AND MATERIALS

The analytical methods and necessary calculations were those described previously[6, 7] in studies of normal pregnant women, similar precautions being observed during collection and analysis. Determinations included the water content and specific gravity of plasma and whole blood, the cell volume and hemoglobin content of whole blood, and the concentration of the important acidic and basic constituents of plasma.

RESULTS

Chronic Cardiovascular Renal Disease.—The clinical data on the nine patients in this group are presented in Table I; the blood chemical findings, in Tables I-A and I-B.

Comparison with the average values noted in the blood of normal pregnant women[6, 7] shows little variation, although the hematocrit reading is somewhat elevated and the water in the cells is below normal. By reason of the depression of the chlorides, the average total acid is in the lower range of normal, as

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is the average total base, because of the diminished sodium content. Both variations may be explained by the lowered concentration of sodium chloride.

Toxemia Without Convulsions.—Data on the fifteen patients in this group are presented in Tables II, II-A, and II-B.

The water content of the plasma is slightly higher than in the hypertensive patients, but the cell water values are similar. The total bases and the total acids are both within the ranges found in normal pregnant women.

Toxemia With Convulsions.—Data on the seven patients with convulsive toxemia are presented in Tables III, III-A, and III-B.

The cell volume, the hemoglobin, the specific gravity of whole blood and plasma, and the water content of the plasma are within the ranges found in normal pregnant women, whereas the water content of the blood cells is significantly reduced.

The average values for total acids are in the lower range for normal pregnant women. Evidently the reduced acidity is caused by a diminution of the bicarbonate rather than by a lowering of the chloride concentration. This change more than compensates for the slight increase in the sulfates.

The average values for total base are slightly lower than in normal pregnant women, a finding which is contradictory to the observations of Stander, Eastman, Harrison, and Cadden.[1] The plasma pH values are generally lower than those for normal pregnant women, especially when the blood is drawn shortly after a convulsion.

DISCUSSION

The results generally confirm observations previously reported and support the contention of Kydd, Oard, and Peters[5] that changes in the acid-base balance of the blood in women suffering from the toxemias of late pregnancy are probably the result of the disease, and that therapy should logically be directed at the relief of the symptoms rather than at the correction of the slight, and possibly temporary, alkali deficit by the administration of alkalies.

Edema, which is a recognized component of the clinical syndrome of the late pregnancy toxemias, was manifest in the majority of the patients studied, although its extent varied markedly. The amount of edema appears to be unrelated to the water content of the plasma or whole blood, or to the plasma proteinate. This latter observation appears to be contrary to earlier work, which showed that the plasma protein concentration diminishes with increasing edema. Peters, Wakeman, Eisenman, and Lee[8] have pointed out that the base-binding capacity of the total protein varies with the albumin-globulin ratio, becoming significantly less as the ratio diminishes. It is well recognized that in edema the diminution of the total plasma protein is largely caused by a reduction in albumin. Such a change would lower the ratio and consequently lead to a proteinate value which is too high. Assuming the correctness of this argument, the findings here recorded are in reasonable accord with previous observations. Moreover, it is obvious that if the recorded proteinate values are too high, the recorded values for total acids both in normal pregnant women and in those with toxemia would also be too high, and that the variations should be more marked in the latter group where the reduction of the A/G ratio is greater.

TABLE I

CHRONIC CARDIOVASCULAR RENAL DISEASE

Clinical Data

CASE NO.	HOSPITAL NO.	AGE	PREVIOUS PREGNANCIES	URINARY ALBUMIN	EDEMA	ADMISSION BLOOD PRESSURE (MM.)	RETINITIS	BLOOD NITROGEN RETENTION	METHOD OF DELIVERY	CHILD		REMARKS
										WEIGHT (GM.)	FATE	
18	G-410	22	0	+	Slight	235/140	Toxic	Slight Uric acid 6.2	Spontaneous, induced	480	Stillborn	Retinal edema and hemorrhages
22	G-645	43	6	+++	None	210/140	Arterio-sclerotic	None	Spontaneous, breech	500	Macerated	Generalized arterio-sclerosis
43a	E-6114	32	6	++	Slight	165/95	None	None	Spontaneous, induced	2,600	Survived	May, 1932
43b	E-6114	33	7	+++	Slight	250/150	None	None	Spontaneous	2,200	Survived	Two convulsions shortly after delivery, May, 1933
56	G-4593	32	2	+++	None	200/130	Early renal	None	Breech extraction	365	Macerated	
60	G-4999	28	5	+	Slight	200/150	Hyper-tensive	None	Spontaneous	3,025	Survived	Essential hypertension
74a	G-11423	45	7	++++	Slight	270/150	Toxic and arterio-sclerotic	None	Vaginal hysterot-omy	365	Stillborn	Admitted in coma; several convulsions 2 days before admission
74b												After 20 hours, medical treatment; slightly improved
74c												Two days later blood drawn shortly before delivery. Patient died of puerperal septicemia on ninth postoperative day
79	Out-patient	?	1	++	None							Patient not in hospital
81	H-1834	24	1	++++	Moder-ate	210/140	Renal	None	Cesarean hysterec-tomy	1,730	Survived	Detachment of retinae, essential hypertension
91	J-1409	23	0	+++	Slight	170/130	Neuro-retinitis	None	Spontaneous, induced	2,120	Survived	

TABLE I-A

CHRONIC CARDIOVASCULAR RENAL DISEASE

(Specific gravity, water and hemoglobin concentrations)

CASE NO.	CELL VOLUME (%)	SPECIFIC GRAVITY			WATER			HEMOGLOBIN		
		PLASMA	WHOLE BLOOD	CELLS	PER KG. PLASMA (GM.)	PER KG. WHOLE BLOOD (GM.)	PER KG. CELLS (GM.)	PER 100 C.C. WHOLE BLOOD (mM)	PER KG. CELLS (mM)	PER KG. WATER (mM)
18	40.0	1.0126	1.0388	1.078	922	812	654	8.26	19.15	29.30
22	37.8	1.0132	1.0323	1.062	900	810	668	8.93	21.88	32.80
43a	40.2	1.0199	1.0345	1.055	913	820	685	7.35	17.31	25.25
43b	37.2	1.0180	1.0441	1.087	920	818	665	7.30	17.90	26.90
56	51.0	1.0158	1.0507	1.083	912	776	654	9.41	17.02	26.02
60	39.5	1.0187	1.0411	1.075	912	807	656	8.17	19.24	29.28
74a	52.0	1.0197	1.0507	1.081	908	778	664	9.95	17.70	26.63
74b	43.0	1.0158	1.0443	1.082	932	810	672	8.22	17.66	26.30
74c	41.0	1.0171	1.0436	1.081	921	814	668	8.14	18.36	27.46
79	44.5	1.0175	1.0437	1.088	901	786	651	9.15	18.90	29.00
81	46.0	1.0180	1.0412	1.069	916	793	657	8.68	17.70	26.96
91	42.7	1.0126	1.0427	1.080	918	798	648	9.50	20.60	31.75
Avg.	42.9	1.0166	1.0423	1.077	915	802	662	8.59	18.62	28.14
Normal pregnant	35.4	1.0154	1.0370	1.078	915	827	674	7.00	18.31	27.23

TABLE I-B

CHRONIC CARDIOVASCULAR RENAL DISEASE

Acid-Base Equilibria in Plasma

(Concentrations are reported in terms of milliequivalents per kilogram of water.)

CASE NO.	$[Cl]^-$	$[HCO_3]^-$	[PROTEINATE]$^-$	$[HPO_4]^{--}+[H_2PO_4]^-$	$[SO_4]^{--}$	TOTAL ACIDS	$[Na]^+$	$[Ca]^{++}$	$[K]^+$	$[Mg]^{++}$	TOTAL BASE	pH
18	108.6	20.2	12.8	2.17	0.5	144.3	145.3	4.37	5.48	2.3	157.5	7.26
22	107.8	28.3	18.1	2.74	1.3	158.2	151.2	5.10	5.14	2.0	163.4	7.43
43a	108.6	26.3	14.6	2.01	0.3	151.8	153.7	4.87	4.67	2.0	163.4	7.38
43b												
56	108.0	28.7	15.3	2.12	0.9	155.0	150.8	4.86	4.28	2.0	161.9	7.39
60	108.5	25.2	18.3	2.56	0.9	155.5	148.9	6.35	4.26	2.0	161.5	7.38
74a	104.8	24.6	14.7	2.32	0.7	147.1	151.9	4.97	5.61	1.9	164.4	7.19
74b	102.1	25.2	13.1	2.51	0.7	143.6	144.1	4.75	5.12	2.1	156.1	7.36
74c	104.2	25.5	13.7	1.89	0.7	146.0	149.1	5.02	6.33	2.2	162.6	7.32
79	112.0	26.3	17.3	1.92	0.8	158.3	156.7	5.66	5.78	2.2	170.4	7.48
81	106.0	28.4	17.8	2.98		156.0*	144.8	4.66	5.12	2.4	157.0	7.48
91	109.6	22.8	14.2	1.97		149.4*	155.1	4.50	7.65	2.3	170.0	7.44†
Avg.	107.3	25.6	15.4	2.29	0.8	151.4	150.1	5.01	5.40	2.13	162.6	7.37
Normal pregnant	112.2	24.6	15.8	2.35	0.55	155.5	151.6	5.15	5.10	2.35	164.1	7.39

*A value of 0.8 was assumed for $[SO_4]^{--}$.

†Determined colorimeterically and corrected to electrometric values by subtracting a determined value of 0.34.

The average values for plasma pH show no marked variation, but there is a significant diminution in those women with convulsive toxemia (eclampsia). Tables III and III-B show that the lowest values were obtained shortly after convulsions. For example, in Case 80, where the blood sample was drawn three minutes after the last (seventh) convulsion, the pH was 7.04, whereas in Case 17 b, a value of 7.22 was obtained several hours after the fifth attack, while the patient was still comatose. In these instances the low pH was associated with

TABLE II

TOXEMIA WITHOUT CONVULSIONS

CASE NO.	HOSPITAL NO.	AGE (YEARS)	PREVIOUS PREGNANCIES	URINARY ALBUMIN	EDEMA	ADMISSION BLOOD PRESSURE (MM.)	RETINITIS	BLOOD NITROGEN RETENTION	METHOD OF DELIVERY	CHILD		REMARKS
										WEIGHT (GM.)	FATE	
23	G-997	26	0	++++	0	175/105	None	None	Spontaneous	1,450	Stillborn	
28	F-8751	32	0	+	Slight	170/110		None	Vaginal cesarean	1,940	Survived	Hemiatrophy of body
34	G-2223	30	4	+	Moderate	135/90			Spontaneous	3,640	Survived	Mild toxemia
46	G-3676	41	1	++	Moderate	170/100	None	None	Spontaneous	1,545	Survived	
48	G-2686	16	0	+	Slight	170/100			Spontaneous	3,900	Survived	
49	G-3929	36	4	++++	Slight	200/110	None	None	Spontaneous	2,645	Survived	
67	G-6109	40	5	+	Marked	180/90	None		Spontaneous, induced	2,175	Stillborn	Marked edema of fetus and placenta
72	G-10567	33	0	+++	Moderate	160/100	None		Cesarean section	2,220	Survived	Marked edema of vulva
73	G-11278	30	1	+++	Slight	200/140	None	Uric acid 5.4 mg.	Spontaneous	2,400	Died on third day	
77a	G-11913	21	0	++++	Moderate	155/115	None	None	Spontaneous	2,640	Macerated	One convulsion 3 days later (see 77b on Table III)
83	H-3412	17	0	++++	Marked	148/100	None	Uric acid 8.4 mg.	Cesarean section	1,560 1,600	Both died on first day	Marked edema of vulva
89	D-5247	25	0	+++	Slight	165/115		None	Spontaneous, induced	2,175	Survived	
90	H-13800	32	3	+	Marked	180/95		None	Spontaneous, induced	2,990	Survived	Hemolytic icterus
95	J-6209	33	9	+++	Moderate	240/140	None	None	Breech extraction, induced	1,550	Died on first day	Hypertension persisted after delivery. Chronic nephritis?
96	J-6504	34	2	+++	Marked	195/100	None	None	Spontaneous, induced	735	Stillborn	Hyperthyroidism, anencephalic monster

TABLE II-A

TOXEMIA WITHOUT CONVULSIONS

(Specific gravity, water and hemoglobin concentrations)

CASE NO.	CELL VOLUME (%)	SPECIFIC GRAVITY			WATER			HEMOGLOBIN		
		PLASMA	WHOLE BLOOD	CELLS	PER KG. PLASMA (GM.)	PER KG. WHOLE BLOOD (GM.)	PER KG. CELLS (GM.)	PER 100 C.C. WHOLE BLOOD (MM)	PER KG. CELLS (MM)	PER KG. WATER (MM)
23	38.0	1.0100	1.0410	1.093	914	808	648	8.34	20.08	31.20
28	35.0	1.0165	1.0330	1.058	908	820	664	7.81	21.10	31.80
34	39.2	1.0078	1.0395	1.089	910	806	657	9.02	21.12	32.20
46	38.5	1.0095	1.0244	1.048	927	826	672	7.85	19.45	28.95
48	32.0	1.0142	1.0345	1.079	924	844	686	6.20	17.95	26.17
49	26.5	1.0149	1.0352	1.091	924	842	628	5.98	20.65	32.88
67	35.8	1.0122	1.0383	1.086	930	835	669	6.65	17.10	25.58
72	42.0	1.0158	1.0416	1.078	920	807	658	8.46	18.70	28.40
73	44.0	1.0120	1.0431	1.068	927	793	633	8.54	18.20	27.10
77a	41.5	1.0130	1.0410	1.081	914	810	670	8.44	18.80	28.10
83	36.7	1.0098	1.0389	1.090	928	827	662	7.08	17.70	26.70
89	42.0	1.0192	1.0446	1.080	914	801	655	8.82	19.42	29.70
90	27.5	1.0184	1.0366	1.086	910	855	713	4.25	14.22	20.00
95	46.0	1.0121	1.0459	1.088	908	783	641	10.12	20.21	31.55
96	29.0	1.0125	1.0291	1.065	928	854	681	6.12	19.75	29.00
Avg.	36.9	1.0132	1.0378	1.079	919	821	662	7.59	18.96	28.62
Normal pregnant	35.4	1.0154	1.0370	1.078	915	827	674	7.00	18.31	27.23

TABLE II-B

TOXEMIA WITHOUT CONVULSIONS

Acid-Base Equilibria in Plasma

(Concentrations are reported in terms of milliequivalents per kilogram of water.)

CASE NO.	$[Cl]^-$	$[HCO_3]^-$	$[PROTEINATE]^-$	$[HPO_4]^{--}+[H_2PO_4]^-$	$[SO_4]^{--}$	TOTAL ACIDS	$[Na]^+$	$[Ca]^{++}$	$[K]^+$	$[Mg]^{++}$	TOTAL BASE	pH
23	109.6	28.0	16.4	2.47	0.4	156.9	149.5	5.50	4.71	2.2	161.9	7.24
28	109.7	24.1	16.7	2.56	0.6	153.7	144.5	4.26	6.36	2.6	157.7	7.42
34	113.6	23.7	16.6	2.43	0.4	156.7	167.3‡	6.43	4.76	2.5	181.0‡	7.37
46	113.0	22.7	14.8	2.47	0.3	153.3	147.7	4.98	4.90	1.9	159.5	7.26
48	112.2	24.6	16.5	2.26	0.9	156.5	156.4	4.98	5.44	1.9	168.7	7.30
49	116.4	24.3	16.0	2.64	0.2	159.5	156.7	5.42	5.03	2.5	169.7	7.30
67	117.9	18.5	16.0	2.95	1.5	156.9	147.0	5.39	5.72	1.9	160.0	7.35
72	111.4	21.9	16.4	2.82	0.7	153.2	161.2	5.00	6.28	1.9	174.4	7.42
73	108.2	27.0	15.6	2.50	1.0	154.3	152.7	5.00	5.18	2.1	165.0	7.37
77a	112.8	23.9	15.2	2.63	0.7	155.2	158.8	5.19	5.60	2.0	171.6	7.41
83	108.8	17.3	17.2	3.10		147.2†	146.0	4.64	7.26	2.3	160.2	7.28
89	111.1	23.3	14.2	2.56		152.0†	147.6	4.60	5.34		159.7*	7.16+
90	114.0	18.5	16.6	2.27		152.2†	151.0	5.06	5.45		163.7*	7.28+
95	102.2	26.6	18.1	3.89		151.6†	146.9	5.16				7.44+
96			15.9									
Avg.	111.5	23.2	16.1	2.68	0.7	154.2	151.2	5.12	5.54	2.2	164.3	7.33
Normal pregnant	112.2	24.6	15.8	2.35	0.55	155.5	151.6	5.15	5.10	2.35	164.1	7.39

+Determined colorimetrically and corrected to electrometric values by subtracting a determined value of 0.34.

*A value of 2.2 was assumed for [Mg] + +.

†A value of 0.8 was assumed for $[SO_4]$ – –.

‡Not included in the average.

TABLE III

TOXEMIAS WITH CONVULSIONS

CASE NO.	HOSPITAL NO.	AGE	PARITY	URINARY ALBUMIN	EDEMA	ADMISSION BLOOD PRESSURE (MM.)	RETINITIS	BLOOD NITROGEN RETENTION	METHOD OF DELIVERY	CHILD		REMARKS
										WEIGHT (GM.)	FATE	
17a	G-23	20	0	+++	Slight	166/130†	Retinal edema only	None; uric acid 5.7	Low forceps and episiotomy	2700	Survived	Blood drawn 2 hours after the fifth convulsion. Patient undelivered
17b					Slight	155/108*						Blood drawn 12 hours, after delivery. Patient in coma; no more convulsions
44	G-3443	18	0	++	Slight	170/90	Normal fundi	None; uric acid 4.5	Spontaneous	1850	Stillborn	One convulsion. Premature separation of the placenta. Acquired syphilis, latent
77b	G-11913	21	0	+++	Moderate	185/120	Normal fundi	None	Spontaneous	2640	Stillborn, macerated	One convulsion 45 minutes before blood was drawn

80	Out-patient case‡	?	0	+	Moderate	170/110	?	?	?	?	?	One convulsion before and six after delivery. Blood drawn 3 minutes after last convulsion
84	Out-patient case‡	?	3	++++	Moderate	166/108	?	?	?	?	?	Two convulsions. Blood drawn 12 hours after second
92	J-5215	21	2	+	Slight	180/100	Normal fundi	No note	Spontaneous	3518	Survived	One post-partum convulsion. Blood drawn 4 hours later
93	J-5370	42	3	++++	Slight	160/100	Small retinal vessels show hypertension. Right retina edematous	Slight; N. P. N. 46 mg.	Spontaneous	850	Stillborn, macerated	Seven convulsions. Blood drawn 1½ hours after last convulsion. Cyanotic with stertorous respiration

*After venesection of 140 c.c. (20 min. later) blood pressure was 140/95; 40 min. later it was 138/90.

†Blood pressure was 125/80 after the convulsion, 30 gr. chloral hydrate was given by rectum after fourth convulsion. One and one-half hours later blood pressure was 142/110; patient was irrational; then ½ hr. later the blood was drawn, and 50 c.c. of 50 per cent glucose were given intravenously. Patient soon became rational.

‡Blood collected in out-of-town hospitals. Little clinical data available.

TABLE III-A

TOXEMIA WITH CONVULSIONS

(Specific gravity, water and hemoglobin concentrations)

CASE NO.	CELL VOLUME (%)	SPECIFIC GRAVITY			WATER			HEMOGLOBIN		
		PLASMA	WHOLE BLOOD	CELLS	PER KG. PLASMA (GM.)	PER KG. WHOLE BLOOD (GM.)	PER KG. CELLS (GM.)	PER 100 C.C. WHOLE BLOOD (MM.)	PER KG. CELLS (MM.)	PER KG. WATER (MM.)
17a	31.8									
17b	34.2	1.0128	1.0296	1.056	913	817	636	6.61	18.31	28.78
44	38.5	1.0141	1.0377	1.074	913	817	672	7.14	17.22	25.63
77b	38.2	1.0068	1.0359	1.081	915	813	659	7.70	18.62	28.30
80	39.9	1.0109	1.0388	1.080	912	806	656	8.11	18.80	28.60
84	39.0	1.0144	1.0297	1.053	918	822	677	8.48	20.60	30.40
92	31.5	1.0170	1.0377	1.085	916	835	666	6.38	18.65	28.00
93	41.0	1.0160	1.0459	1.090	918	801	645	9.88	19.85	30.80
Avg.	37.5	1.0133	1.0364	1.074	915	816	659	7.76	18.86	28.60
Normal pregnant	35.4	1.0154	1.0370	1.078	915	827	674	7.00	18.31	27.23

TABLE III-B

TOXEMIA WITH CONVULSIONS

Acid-Base Equilibria in Plasma

(Concentrations are reported in terms of milliequivalents per kilogram of water.)

CASE NO.	$[Cl]^-$	$[HCO_3]^-$	$[PROTEINATE]^-$	$[HPO_4]^{--}+[H_2PO_4]^-$	$[SO_4]^{--}$	TOTAL ACIDS	$[Na]^+$	$[Ca]^{++}$	$[K]^+$	$[Mg]^{++}$	TOTAL BASE	pH
17a*	115.5		14.2	2.50	2.6		142.5	3.79	5.38	2.7	154.4	7.19
17b	115.7	15.3	13.5	1.78	2.6	148.9	143.8	3.76	5.54	2.7	155.8	7.22
44	107.0	23.2	18.5	2.48	0.2	151.4	155.4	5.19	4.69	2.0	165.5	7.34
77b	112.2	18.2	13.1	2.91	1.5	147.9	155.9	4.89	5.46	2.4	168.7	7.27
80	119.5	14.4	11.7	3.30	0.4	149.3	162.1	5.31	5.63	2.4	175.4	7.04
84			18.4				137.2	3.92				
92	105.8	27.0	19.2	2.60		155.4‡	140.0	4.65	8.74	2.4	155.8	7.44†
93	106.9	23.6	17.1	2.22		149.8‡	153.8	4.56	5.64	2.3	166.3	7.38†
Avg.	111.8	20.4	15.7	2.54	1.5	150.5	149.0	4.51	5.86	2.4	163.2	7.26
Normal pregnant	112.2	24.6	15.8	2.35	0.55	155.5	151.6	5.15	5.10	2.35	164.1	7.39

*Concentrations were calculated on the basis of the water content of the blood in 17b.

†Determined colorimetrically and corrected to electrometric values by subtracting a determined value of 0.34.

‡A value of 0.8 was assumed for $[SO_4]^{--}$.

a marked reduction of the bicarbonate. While no determinations for lactic acid were made, it seems probable that these observed changes toward the acid side were due to an accumulation of lactic acid developed in the organism as a result of the convulsive seizures, as emphasized by Stander.[9, 10]

SUMMARY

The acid-base balance in the blood has been studied in thirty-one women suffering from various forms of the toxemia of late pregnancy (nine hypertensive, fifteen nonconvulsive, and seven eclamptic patients). In general, the findings confirm the work of previous investigators by demonstrating a slight alkali deficit, without any significant change in the hydrogen-ion concentration, except in eclampsia, where a convulsive seizure is followed by a marked reduction in the bicarbonate and an associated, proportional lowering of the pH.

On the basis of accumulated evidence, it would appear that the altered acid-base relationships of the blood in patients with pregnancy toxemia are of minor importance, and that any serious metabolic disturbances accompanying this disease entity must be sought in some tissue other than the blood.

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EFFECT OF DIET ON ARTERIAL AND VENOUS GLUCOSE TOLERANCE CURVES IN RHEUMATOID ARTHRITIS*

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CHRONIC rheumatism is the center of considerable investigative work since it is one of the chief diseases causing partial or total disability in man. Rheumatoid arthritis is a type of chronic arthritis which is especially important economically since it attacks the young adult, as a rule, and converts an economic asset into a liability.[1-4] Considerable investigative work has been done in an effort to explain the causes, as well as changes, that occur in the body of the patient afflicted with this disease. The possibility of some derangement in the metabolic or endocrine system of the body has been considered as a possible etiologic factor;[5-8] however, chronic infection seems to interest the greatest number of investigators.[11-13]

Regardless of etiology, certain alterations in body economy are said to exist in the rheumatoid types of arthritis; there is a loss of muscle bulk, diminished circulation, and gastrointestinal stasis. Certain alterations in the chemistry of the body are also said to occur. A diminution in the blood content of vitamins B and C has been reported,[9, 10] and there is said to be a diminished tolerance for glucose.[14]

Pemberton and others have shown that the arthritic person does not remove glucose from the blood as promptly as the normal individual. Because of the apparent reduction in carbohydrate metabolism, many investigators recommend low carbohydrate diets for the rheumatoid arthritic patient and have reported marked relief from pain when patients are placed on a high fat, low carbohydrate diet.

The experience of our clinic has been similar to that of Pemberton and others, in that the majority of the patients studied had a decreased tolerance for dextrose. We have felt that this diminished tolerance might also be explained by an impairment in the glycogenic function of the liver. If this is the case, improvement in the carbohydrate metabolism would be expected with a high carbohydrate rather than a high fat diet. Since glucose tolerance tests done on venous blood give only an idea of the blood sugar after the blood has coursed through the muscles, it was thought that a better idea regarding the ability of the liver to remove glucose from the blood would be obtained by the simultaneous estimation of both arterial and venous blood. In this way the level of the blood sugar could be ascertained after the blood has passed through the

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liver but not through the muscles. If the arterial blood sugar is high, the ability of the liver to remove sugar from the blood is impaired. On the other hand, if both the venous and arterial blood sugars are high and tend to approximate each other, the glycogenic function of both liver and muscle is impaired, since, when muscle function is good, the arteriovenous difference should be marked. It has been reported by Sweeney, and confirmed by others,[16] that a high carbohydrate diet improves carbohydrate utilization in normal individuals. It was felt that it would be of value to determine whether: (1) diet had the same effect on the glucose tolerance of arthritic patients as that of normal individuals; (2) improvement followed one of the two diets used, whether it was due to improved liver or muscle function; (3) what the effect of the respective diets on the well-being of the patient might be.

PROCEDURE

Patients with typical rheumatoid arthritis were selected for the study with each patient showing varying degrees of muscle wasting and joint involvement of from two to twelve years' duration. Each patient was carefully examined for foci of infection. Blood nonprotein nitrogen, sugar, cholesterol, uric acid, Wassermann, and gonococcal complement fixation determinations were studied. Complete blood counts were done and chest x-ray studies were made.

Eleven patients, eight males and three females, were found to be suitable for this study; all had normal values for the above determinations. Each patient remained afebrile during the study and only occasionally received codeine with salicylates as symptomatic treatment. Three normal subjects, two females and one male, were also selected for the same study and acted as controls. Those selected were hospitalized and placed first on a high fat, low carbohydrate diet for two weeks; then on a high carbohydrate, low fat diet for another period of two weeks. At the end of this period the patient remained on a high carbohydrate diet but was given 10 units of insulin before each meal for a third period of two weeks. At the end of each period complete arteriovenous glucose tolerance tests were run on each patient, the arterial and venous bloods being collected at the same time. The various diets were equicaloric and contained an equal amount of protein. The high carbohydrate diet contained from 350 to 450 Gm. of carbohydrate and from 40 to 50 Gm. of fat, while the high fat diet contained from 200 to 225 Gm. of fat, and from 70 to 80 Gm. of carbohydrates.

The glucose tolerance test was run over a three-hour period. Following the collection of the fasting specimen of blood, a solution containing 100 Gm. of dextrose was taken by mouth. Specimens of blood were obtained from the cubital vein after thirty minutes, sixty minutes, two hours, and three hours. The arterial blood was collected from the finger tip simultaneously with the venous puncture, since it has been shown that cutaneous and arterial blood are identical in sugar values.[17]

RESULTS

Table I represents the results obtained in the three normal individuals. It will be noted that a lowering in the blood sugar curves occurred following a high carbohydrate diet. The glucose tolerance curve was more elevated after

the patient had been given insulin, plus a high carbohydrate diet, than it was when the patient was on a high carbohydrate diet, but the elevation was less than when the high fat diet was taken.

In the patients listed in Table II only venous sugars were obtained. It will be noted that the venous levels following a high fat diet reached almost diabetic proportions; however, following a high carbohydrate diet, the curves were considerably flattened.

TABLE I

PATIENT	DIET	MG. % SUGAR	TIME—MINUTES					AVG. DIFF. AT 30 MIN. (MG. %)
			FASTING	30	60	120	180	
M. M. F—20 yr.	High fat	Arterial	80	200	142	78	124	
		Venous	76	165	118	69	114	35
	Low fat	Arterial	83	128	106	69	101	
		Venous	74	113	88	58	85	15
	After insulin	Arterial	94	185	99	117	106	
		Venous	81	140	88	98	89	45
P. G. F—21 yr.	High fat	Arterial	70	145	156	115	50	
		Venous	69	103	109	87	49	46
	Low fat	Arterial	80	151	156	106	110	
		Venous	73	121	111	74	80	45
	After insulin	Arterial	85	170	164	115	106	
		Venous	73	138	134	94	84	32
R. A. M—28 yr.	High fat	Arterial	101	133	138	101	83	
		Venous	80	116	85	85	76	52
	Low fat	Arterial	85	112	106	100	88	
		Venous	82	110	80	87	78	26
	After insulin	Arterial	82	140	110	110	100	
		Venous	76	123	105	96	80	17

TABLE II

TIME	R. L. R. 32 YR.—FEMALE			J. L. B. 37 YR.—MALE			R. R. B. 28 YR.—MALE		
	HIGH FAT	LOW FAT	AFTER INSULIN	HIGH FAT	LOW FAT	AFTER INSULIN	HIGH FAT	LOW FAT	AFTER INSULIN
	VENOUS BLOOD MG. %			VENOUS BLOOD MG. %			VENOUS BLOOD MG. %		
Fasting	99	96	87	85	75	71	103	81	81
30	187	185	147	151	107	154	183	112	134
60	208	129	223	170	160	165	188	134	156
120	229	118	171	76	71	134	189	103	125
180	187	75	112	52	64	113	132	67	61

Both arterial and venous blood sugars were determined simultaneously in the eight patients listed in Table III. The tolerance is much diminished following a high fat diet, but is improved following a high carbohydrate diet. The effect of insulin is the same as in the normal individuals and points to a definite diminution in tolerance. Comparison of the arterial and venous blood sugars reveals a definite flattening of both the arterial and venous curves following a high carbohydrate diet, with a greater effect on the arterial curve. In four of the eight patients there occurred an increase in the arteriovenous difference following the use of a high fat diet.

Chart I was obtained by plotting the average values of both arterial and venous blood sugars so that an idea of the effect of diet and insulin might be ob-

TABLE III

PATIENT	DIET	MG. SUGAR	TIME—MINUTES					AVG. DIFF.
			FASTING	30	60	120	180	
H. R.	High fat	Arterial	95	200	210	140	128	38
31 yr.		Venous	92	154	169	107	94	
Male	Low fat	Arterial	97	160	180	160	130	38
		Venous	89	140	138	105	89	
	Insulin	Arterial	108	148	162	169	98	24
		Venous	79	138	134	121	79	
V. B.	High fat	Arterial	108	142	183	144	97	22
36 yr.		Venous	73	134	145	125	95	
Male	Low fat	Arterial	86	132	124	116	85	9
		Venous	82	128	118	100	80	
	Insulin	Arterial	83	156	186	164	99	9
		Venous	78	154	175	151	97	
C. M.	High fat	Arterial	75	178	187	142	118	28
29 yr.		Venous	76	158	141	109	105	
Male	Low fat	Arterial	88	138	170	138	116	24
		Venous	83	131	134	103	101	
	Insulin	Arterial	74	144	192	168	160	25
		Venous	71	123	156	154	128	
A. M.	High fat	Arterial	88	218	227	174	128	54
28 yr.		Venous	78	154	156	123	98	
Male	Low fat	Arterial	97	151	135	88	78	22
		Venous	88	114	107	71	71	
	Insulin	Arterial	88	192	196	192	119	28
		Venous	80	174	158	158	92	
N. B.	High fat	Arterial	90	186	210	140	122	33
28 yr.		Venous	80	140	156	120	110	
Male	Low fat	Arterial	95	174	138	128	122	23
		Venous	80	143	100	99	88	
	Insulin	Arterial	92	186	169	115	98	20
		Venous	91	158	145	100	85	
R. H.	High fat	Arterial	75	144	220	219	192	15
27 yr.		Venous	71	138	199	194	185	
Female	Low fat	Arterial	78	180	200	164	140	17
		Venous	75	158	188	150	118	
	Insulin	Arterial	90	148	215	210	189	12
		Venous	88	140	200	194	180	
S. J.	High fat	Arterial	88	188	221	193	162	31
32 yr.		Venous	81	158	190	180	112	
Male	Low fat	Arterial	89	145	123	109	95	7
		Venous	85	138	118	100	92	
R. J.	High fat	Arterial	75	142	190	180	147	18
26 yr.		Venous	71	129	180	173	124	
Female	Low fat	Arterial	88	167	189	94	98	10
		Venous	86	161	179	83	88	

tained at a glance. The flattening of both arterial and venous curves in the normal individual following the high carbohydrate diet is striking. Following insulin, the curve resembles that obtained by a high fat diet. It will be noted that the arthritic person tends to have a higher blood sugar following a carbohydrate test meal than does the normal individual; nevertheless, the response to a high carbohydrate diet, as well as to insulin, is similar to the effect produced in the normal individual. Following a high carbohydrate diet, the arterial curve of the arthritic person approaches that of the normal, indicating an improved utilization of sugar by the liver.

DISCUSSION

The result of our work demonstrates that a diminished tolerance for carbohydrates exists in rheumatoid arthritis, as was reported by Pemberton and

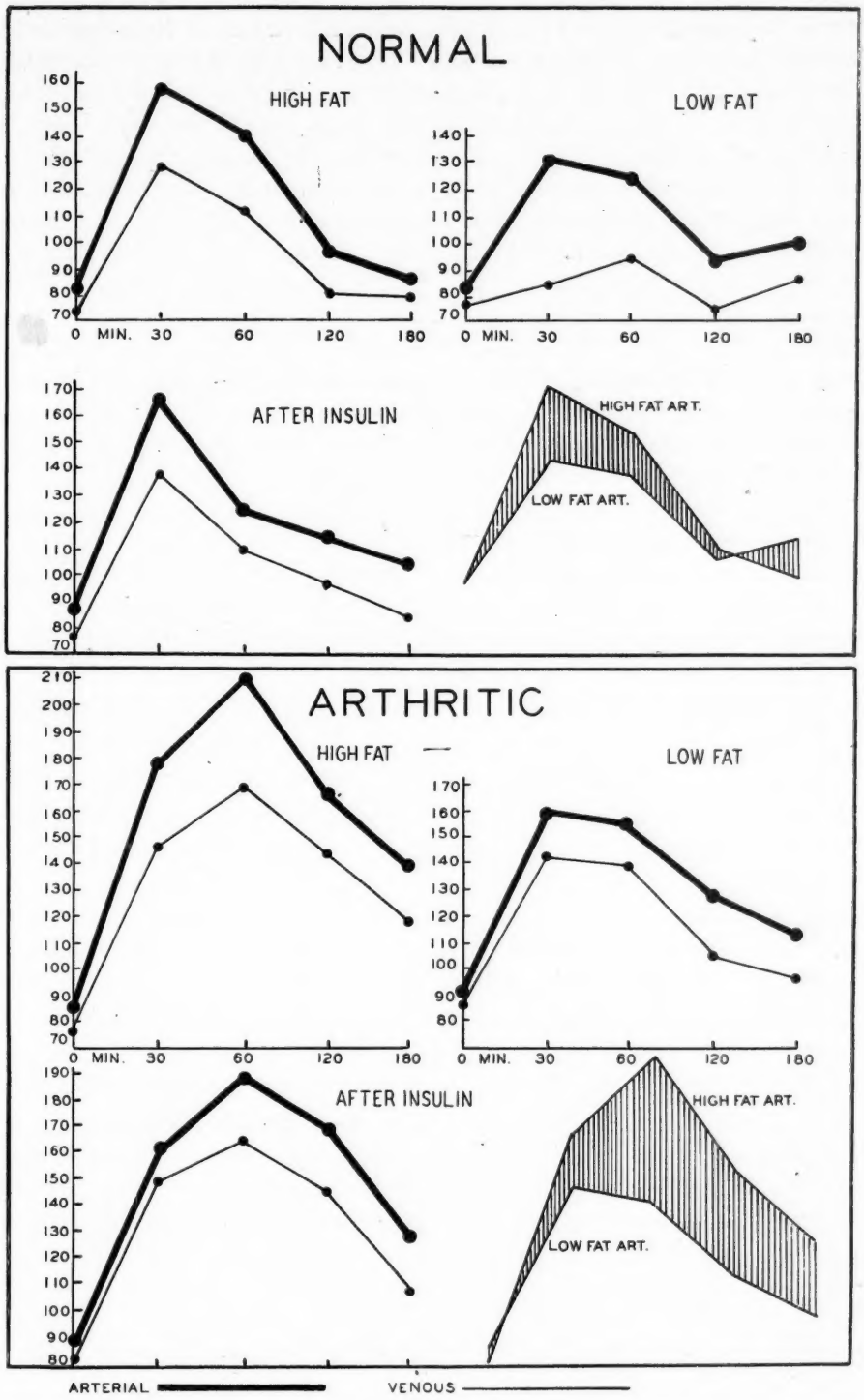
NORMAL
HIGH FAT
LOW FAT
AFTER INSULIN
HIGH FAT ART.
LOW FAT ART.
ARTHRITIC
HIGH FAT
LOW FAT
AFTER INSULIN
HIGH FAT ART.
LOW FAT ART.
0 MIN. 30 60 120 180
ARTERIAL
VENOUS

Chart 1.

others. Following a two weeks' course of insulin, these patients have a decreased tolerance to glucose, as well as a diminution in arteriovenous difference similar to those exhibited by our normal controls. This only adds to common knowledge that insulin is diabetogenic when given to a person without diabetes over a long period of time. Various explanations have been offered to account for the diminished tolerance to glucose: (a) disturbance of pancreatic function, (b) diminished circulation causing a slowing in the removal of sugar from the blood, and (c) hepatic dysfunction. From the evidence submitted, it would seem that the most important role is played by the liver. This is shown by (1) the improvement following the high carbohydrate diet, (2) a decrease in arterial blood sugar levels following a carbohydrate test meal, and (3) no loss in glycogenic function of the muscles. The evidence presented does not, we believe, aid materially in the determination of the etiology of this disease. Chronic infection may produce metabolic changes similar to those seen in these patients. But other conditions, such as avitaminosis and endocrine disturbances, may likewise produce a similar picture.

Although insulin seemed to decrease the tolerance to glucose, an improvement in appetite of all patients was noted. No definite gain in weight and no effect on the course of the arthritis were demonstrated. None of the patients studied complained of an increase in pain while on the high carbohydrate diet. There was no evidence of increased pathologic activity within the joint during this regime. The opposite was true, however, in that the patients experienced a sense of well-being and frequently asked that they be kept on a high carbohydrate diet.

SUMMARY AND CONCLUSIONS

1. The effects of a high fat diet, a high carbohydrate diet, and a high carbohydrate diet with insulin on the tolerance to dextrose was observed in three normal persons and in eleven patients with rheumatoid arthritis.

2. Simultaneous arterial and venous true sugar tolerance curves were obtained on three normal persons and on eight with arthritis. Venous curves alone were run on three persons with arthritis.

3. The tolerance to glucose was improved in all persons with arthritis and in the normal person after a high carbohydrate diet.

4. Insulin decreased the tolerance to glucose in all patients studied.

5. The evidence submitted suggests that the decreased tolerance to glucose in the individual with arthritis is due to decreased glycogenic liver function rather than to pancreatic dysfunction or circulatory stasis.

6. There was no evidence of increased pain or increased disability in the person with arthritis while on the high carbohydrate regime.

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VARIATIONS IN THE COMPOSITION OF GASTRIC JUICE*

Influence of Protein on the Acidity and Pepsin

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THE purpose of the present series of investigations is to study systematically the effect of proteins, carbohydrates, and fats on the acidity and pepsin activity (at constant pH) of the gastric secretions.

The hitherto available data on the *simultaneous* study of the pepsin activity, pH, and free and total acidity are scarce. This is especially true for the pepsin method of Anson and Mirsky[1] and the hydrogen-ion determination by the more reliable potentiometric methods. There are no data available for studying the influence of the three main food classifications into which diets may be divided in conjunction with the sensitive double histamine test proposed by Rivers, Osterberg, and Vanzant.[2]

The report presented here gives the results of the protein study. The protein used was gelatin. It was selected because of its purity, availability, ease of administration, and solubility in the gastric contents.

METHODS

All observations were made on patients who gave clinical and x-ray evidence of peptic ulcer.

The procedure for carrying out the test meal was as follows: The patient presented himself in the fasting state. A Rehfuss tube was introduced into the stomach, and the fasting contents were withdrawn. Five-tenths milligram of histamine hydrochloride was then injected hypodermically, and immediately afterwards 120 c.c. of tap water were given orally. Five to ten cubic centimeter samples of gastric juice were then aspirated at fifteen-minute intervals for one hour. At the end of the hour the entire stomach contents were removed and 0.5 mg. of histamine hydrochloride was again injected. Immediately afterward, 120 c.c. of tap water were given orally. When the effect of the protein was to be determined, a suspension of 15 Gm. of U.S.P. gelatin in 120 c.c. of tap water was given. Five to ten cubic centimeter samples of gastric juice were then aspirated at successive fifteen-minute intervals for one hour. The patient was told to expectorate his saliva throughout the test.

After collection each sample was placed in the refrigerator. The samples in most instances were free from mucus and did not require filtering. The pH

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and the free and total acidity were determined within two hours after collection of the last sample. Pepsin determinations were done the following morning on a portion of the gastric juice diluted with 0.1 normal hydrochloric acid and kept in the refrigerator overnight. Pepsin values were found to be constant under these conditions.

The pH determinations were made with the Beckmann glass electrode. Free and total acidities were determined in the usual manner by titration. Values are expressed in terms of milliequivalents (meq.) per 1,000 c.c. These are numerically equivalent to what is commonly referred to as free and total acidity. The pepsin content was determined by the method of Anson and Mirsky[1] following the procedure outlined by Mullins and Flood.[3] Pepsin values are expressed as milligrams of pepsin (1:4,000) per cubic centimeter of gastric juice, as suggested by Helmer, Fouts, and Zerfas,[4] and Mullins and Flood.[3] In carrying out the pepsin estimations, all dilutions were made with 0.1 normal hydrochloric acid.

Influence of Protein on the pH, Pepsin, and Free and Total Acidity of Gastric Juice During a Modified Double Histamine Test.—In testing the effect of the protein on the gastric secretions in vivo, a group of 16 patients with peptic ulcer were given the double histamine test without the protein (water group) after the first hour, and a separate group of 19 patients with peptic ulcer were given the double histamine test with the protein after the first hour (protein group).

Table I presents the mean values of pH, pepsin, and free and total acidity for these two groups. The significance of the difference between the mean values were evaluated by the statistical methods of Fisher[5] as applied to small samples. P represents the frequency with which the difference between two means may be due to chance alone. When P is 0.05 or less, the difference between the means is considered significant.

It can be seen in Table I that during the first hour no statistically significant differences exist between the mean pH, pepsin, and free and total acidity values for the two groups. During the second hour, when one group received protein in addition to water, statistically significant differences can be noted between the mean pH, pepsin, and free acidity values for the first forty-five minutes. The total acidity values show no statistically significant changes during this period.

Thus protein in the form of gelatin when introduced into the stomach raises the pH markedly for at least thirty minutes and noticeably for forty-five minutes, markedly lowers pepsin and free acidity of the stomach for thirty minutes and noticeably for forty-five minutes, but has no effect on the total acid concentration.

Influence of the Protein on pH, Pepsin, and Free and Total Acidity in Vitro.—Since the concentration of the various constituents of the gastric juice depends on both chemical and secretory mechanisms, in vitro experiments testing the purely chemical effect of protein in the form of gelatin on acidity and pepsin were also carried out. The results are presented in Table II.

It can be seen in Table II that the protein (gelatin) markedly reduces the free acidity and causes a definite increase in the pH and a slight increase in the total acidity values. These findings are due to the acid-combining properties of

TABLE I

INFLUENCE OF PROTEIN (GELATIN) ON THE ACIDITY AND PEPSIN OF GASTRIC JUICE

	INGESTED MATERIAL	MEAN VALUES: TIME INTERVAL AFTER FIRST INJECTION OF HISTAMINE (MIN.)				INGESTED MATERIAL	TIME INTERVAL AFTER SECOND INJECTION OF HISTAMINE (MIN.)			
		15	30	45	60		15	30	45	60
Free acidity*	Water	35	54	61	63	Water	50	67	69	69
	Water	31	49	55	58	Water + protein	11	27	42	51
P values	+	0.62	0.68	0.48	0.68		10^{-5}	$<10^{-5}$	0.02	0.11
Total acidity*	Water	48	69	77	79	Water	61	81	85	86
	Water	48	65	74	79	Water + protein	55	69	79	84
P values		1.00	0.72	0.77	1.00		0.57	0.25	0.56	0.87
pH	Water	1.97	1.41	1.32	1.30	Water	1.39	1.26	1.23	1.25
	Water	1.83	1.47	1.39	1.40	Water + protein	3.25	2.57	1.97	1.69
P values		0.78	0.62	0.54	0.44		$<10^{-6}$	10^{-3}	0.035	0.11
Pepsin values†	Water	4.9	5.7	6.1	6.6	Water	5.9	6.2	6.3	6.8
	Water	4.8	5.3	5.8	5.8	Water + protein	3.6	3.8	4.8	5.9
P values		0.84	0.64	0.67	0.32		$<10^{-3}$	$<10^{-3}$	0.05	0.25

*Expressed in milliequivalents per 1,000 c.c. of gastric juice. Numerically, this corresponds to the units used clinically.

†Pepsin values are expressed as milligrams of pepsin (1:4,000) per cubic centimeter of gastric juice. P represents the frequency with which the difference between the two means may be due to chance alone.

the protein on the acid side of the isoelectric point and to the base-combining power on the alkaline side of the isoelectric point. The presence of the protein has no effect on the pepsin values, as determined by the method of Anson and Mirsky.[1]

The possibility that pepsin may be destroyed when the pH of a pepsin solution is raised by addition of the protein and incubated at 37° C. for an hour was then investigated . It was found that raising the pH from 1.3 to 4.0 by the addition of the protein and thereafter incubating at 37° C. has no effect on the peptic activity, as determined by the Anson-Mirsky method.

TABLE II

INFLUENCE OF PROTEIN (GELATIN) ON THE ACIDITY AND PEPSIN IN VITRO

PROTEIN ADDED GM. PER 1,000 C.C. OF SOLUTION	FREE ACIDITY MEQ. PER 1,000 C.C.		TOTAL ACIDITY MEQ. PER 1,000 C.C.		pH			PEPSIN MG. (1:4,000) PER C.C.
0	42	91	50	100	1.0	1.3	1.6	6.3
10					1.0	1.4	1.8	6.3
20	27	76	53	103				
30								6.1
50	5	53	57	107	1.3	2.5	4.0	6.2
80					1.6	3.7	4.5	
100	0	9	64	113	2.1	4.0	4.6	6.3
120					2.9	4.2	4.7	
150	0	0	70	120	3.5	4.4	4.9	

The possibility that the pepsin digestion products of the protein would reduce peptic activity was also investigated. It was found that pepsin activity remained constant when the protein is digested at a pH of 3.0 for one hour at 37° C. This is in harmony with Northrop's experiments[6] which show that the digestion of gelatin by pepsin takes place very slowly and then not to any great extent.

DISCUSSION

From the results obtained, it is to be seen that protein in the form of gelatin significantly reduces the hydrogen-ion concentration and pepsin, but not the total acid concentration of the gastric juice. This reduction of the hydrogen-ion concentration in the stomach is similar to the in vitro reduction of acid by the protein. The amount of reduction depends upon the quantity of acid present and the amount of protein added, as shown in Table II.

The effect of the protein on total acidity in vivo is also in harmony with the in vitro findings where (as seen in Table II) the presence of gelatin in small concentrations causes only slight variations in the total acidity. In the in vivo experiments gelatin was given in a concentration of 12 per cent. This concentration is quickly reduced by the addition of fluid from the gastric secretions, so that the concentration present in the stomach is considerably less than 12 per cent. In terms of total acidity this would mean an increase by only a few milli-equivalents per 1,000 c.c. In line with this, no significant differences in the total acid was noted between the protein and control groups.

From the in vitro experiments performed, the mechanism by which the pepsin concentration is lowered in the stomach, in the presence of protein in the

form of gelatin, cannot be ascribed to any competitive retardation of peptic activity by the protein or to the destruction of pepsin when the pH is raised by the protein or to the inhibition of peptic activity by the digestion products of the protein. The factors that are possibly responsible for the lowering of the pepsin content of the gastric juice following the administration of the protein may be either a lowered volume rate of secretion of pepsin or a retardation in the rate of conversion of the pepsin precursor, pepsinogen, to pepsin.

SUMMARY

1. The influence of protein (in the form of gelatin) on the acidity and pepsin of the gastric juice of patients suffering from peptic ulcer was investigated by means of a modified double histamine test.

2. The hydrogen-ion concentration, the free acid, and the pepsin were reduced. The total titrable acidity was not appreciably influenced.

3. On the basis of in vitro experiments the reduction of the hydrogen-ion concentration and free acidity of the gastric juice may be ascribed to the acid-combining power of the protein.

4. The reduction of the pepsin content of the stomach, on the basis of in vitro experiments, cannot be ascribed to the protein per se, but possibly to a reduced secretion of pepsin or a retarded conversion of pepsinogen to pepsin.

We are indebted to Dr. C. A. Flood, of the Columbia College of Physicians and Surgeons, for his helpful suggestions regarding the technique of pepsin estimations.

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A CRITICAL SURVEY OF A PHENOLPHTHALEIN TEST FOR GASTROINTESTINAL LESIONS*

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IN VIEW of the need for a simple test to determine the presence of gastrointestinal lesions quickly and accurately, Woldman[1] devised a procedure based upon the urinary excretion of peroral phenolphthalein when a break exists in the mucosa of the digestive tract.

The patient in a fasting state is given 0.1 Gm. (1.5 gr.) of white phenolphthalein dissolved in 10 c.c. of 95 per cent alcohol, diluted with 20 c.c. of water. Specimens of urine are obtained two and four hours later and tested immediately for the presence of free phenolphthalein by the addition of a 10 per cent solution of sodium hydroxide. Woldman assumes that the appearance of a pink color indicates that free phenolphthalein has actually passed through a break in the continuity of the gastrointestinal mucous membrane into the blood and is finally excreted in the urine. Normally, it is excreted as conjugate phenolphthalein which fails to give this specific reaction. Prolongation of the test beyond four hours did not aid its diagnostic efficiency. Occasionally, the urinary pigments in very dark or highly colored urine presented a problem because of the masking of the faint pink color reaction of the indicator. Greene[2] suggested an improvement on the test by the addition of suspensions of the hydroxides of zinc, calcium, or barium to remove the pigments in the urine and at the same time produce the necessary alkalinization. Comparative tests run concomitantly on such urines showed a more visible pink with these suspensions. However, it required from two to three times the amount of these alkalies to get the necessary alkalinization. Therefore, whenever the test is employed, it is suggested that one of these alkalies be made available for highly colored urines.

Among the advantages of the test are enumerated its usefulness in cases where the roentgenographic diagnosis is doubtful or impossible to perform, in ruling out organic lesions of the gastrointestinal tract in diseases which may have symptoms referable to the digestive system, in differentiating functional from organic disease, and its simplicity, rapidity, and inexpensiveness. Woldman also reports an amazing accuracy for a laboratory test of its kind, with a possible error of less than 3 per cent for both positive and negative findings.

The test proved to have several serious disadvantages in our hands. The percentage of error in our series of cases is much greater than that reported, and, therefore, the test loses much of its specificity. In a series of 46 persons with proved gastrointestinal lesions, 22 failed to give a positive reaction, resulting in

*From the Kings County Hospital, service of Dr. C. H. Greene, and the Long Island College of Medicine Division, Dr. J. Hamilton Crawford, director.
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48 per cent error (Table I). A group of 105 nongastrointestinal control patients gave 25 false positive reactions, resulting in 24 per cent error (Table II). Upon repetition of the test on individuals with false positive reactions, practically the same results were obtained. The total error for both positive and negative findings was 38 per cent. These findings closely approximate the fairly uniform results obtained by other investigators (Table III).

TABLE I

RESULTS OF THE PHENOLPHTHALEIN TEST IN PATIENTS WITH GASTROINTESTINAL LESIONS

DIAGNOSIS	CASES	RESULT	
		POSITIVE	NEGATIVE
Peptic ulcer	12	7	5
Hematemesis	7	5	2
Carcinoma of stomach	9	4	5
Carcinoma of colon	4	1	3
Ulcerative colitis	5	2	3
Tuberculous enteritis	3	2	1
Amebic dysentery	1	0	1
Diverticulitis of colon	2	1	1
Acute ulcerative gastritis	1	0	1
Typhoid	2	2	0
Total	46	24	22

TABLE II

RESULTS OF THE PHENOLPHTHALEIN TEST IN PATIENTS WITHOUT GASTROINTESTINAL LESIONS

DIAGNOSIS	CASES	RESULTS	
		POSITIVE	NEGATIVE
Normal persons	10	1	9
Gall bladder disease	9	2	7
Chronic glomerulonephritis	3	1	2
Resolved pneumonias	14	4	10
Nongastrointestinal Malignancy (e.g., breast, lung, bone, etc.)	5	1	4
Cirrhosis of the liver	4	2	2
Cardiovascular disease (not decompensated)	25	7	18
Blood dyscrasias	5	2	3
Arthritis	7	1	6
Pulmonary tuberculosis	11	2	9
Asthma	5	1	4
Gastric neurosis	1	0	1
Upper respiratory infection	5	2	3
Diabetes	1	0	1
Total	105	26	79

In view of the fact that the tests were performed exactly as outlined by Woldman, guarding against contaminations, and testing specimens immediately following their collection, so that free phenolphthalein could not possibly be formed from the conjugated compound on standing, causes were investigated to explain these wide discrepancies. Especially was an explanation sought for the presence of the many false positive reactions. Notkin, Kirsch, and Albert[3] believe that this is due to the fact that certain catharties (phenolphthalein, rhubarb, senna, and cascara) impart a brown color to the urine which changes to red on the addition of alkalies. Furthermore, urorosein and uroerythrin produce a similar color in an alkaline medium.

TABLE III

AUTHORS	TOTAL NO. OF PATIENTS	PATIENTS WITH KNOWN GASTROINTESTINAL LESIONS			CONTROL PATIENTS WITHOUT GASTROINTESTINAL LESIONS		
		NO. CASES	% POSITIVE	% NEGATIVE (FALSE)	NO. CASES	% POSITIVE (FALSE)	% NEGATIVE
Kremer, D. N., Shore, P. D., and Wiesel, B. H.[19]	137	23	56	44	114	21	79
Suttenfield, D. F.[20]	90	12	17	83	78	23	77
Notkin, L. J., Kirsch, E., and Albert, S.[3]	105	13	70	30	92	37	63
Le Vine, R., and Kirsner, J. B.[21]	115	44	62	38	71	46	54
Slutsky, B., and Wilhelmj, C. M.[22]	14	8*	36	64	6	50	50
Steigmann, F., and Dyniewicz, J. M.[9]	200	56	78	22	144	73	27
Reich, N. E.	151	46	52	48	105	24	76

*Experimentally induced ulcers in dogs.

In an early exhaustive study Fleig[4] proved that phenolphthalein is a stable compound which does not break down in the gastrointestinal tract into phenol and phthalic acid, and when given by mouth in larger doses, passes into the urine in its free state which may be discovered by the addition of alkali. Von Vamossy,[5] who discovered the cathartic quality of phenolphthalein in 1902, found that more than 85 per cent of ingested phenolphthalein is eliminated in the feces of the dog and that a minimal quantity of free phenolphthalein is recovered from the urine, the remainder appearing as conjugated phenolphthalein.

Fantus and Dyniewicz[6] indicated that after small medicinal doses free phenolphthalein is usually absent from the urine, but that the larger the dose, the more frequently does free phenolphthalein appear in the urine. The same authors,[7] in more extensive studies on the elimination of phenolphthalein, demonstrated that three out of seven students receiving approximately the same dose (0.12 Gm.) as that used in the test, eliminated the following percentages of free phenolphthalein of the total dose: 0.1, 0.37, 1.06, respectively. Again, these authors[17] recovered free phenolphthalein in 8.5 per cent of the urines of medical students and in 21.5 per cent of the urines of the patients at the Cook County Hospital without gastrointestinal lesions. Since free phenolphthalein shows its presence as a pink color in alkaline solutions in dilutions as high as 1:12,000,000 (0.1 Gm. to 120 liters),[8] even the excretion of traces, which most investigators agree occurs, can give a positive test in normal persons, or in those without demonstrable organic gastrointestinal lesions. The occurrence of these traces of the free drug in the urine is the most important cause for its failure as a specific test in a large number of negative controls. Fantus and Dyniewicz[6] reverse an earlier conception by concluding that the smaller the dose the larger the relative percentage of free phenolphthalein appearing, probably because the lesser amount of catharsis permits increased absorption. This may account for its urinary presence even with the smaller dosages.

More recently, Steigmann and Dyniewicz[9] believe that there is a certain minimum of conjugated phenolphthalein below which no free phenolphthalein appears in the urine, and this amount is 5 mg. per 100 c.c. Therefore, in corroborating previous investigations,[23] the amount of conjugated phenolphthalein governs the absence or presence of free phenolphthalein. Hence, any condition which will promote an increased formation or circulation in the blood of the conjugated form will lead to the appearance of the free form in the urine. This also contradicts Woldman's assumption that free phenolphthalein depends entirely upon the presence of an ulcerative lesion. While increased intestinal absorption may play a role, the appearance of the free form depends so greatly upon the amount of the conjugated form that it is wrong, in their opinion, to attribute any special diagnostic importance to free phenolphthalein in the urine.

Patients with jaundice show practically 100 per cent free phenolphthalein present and would therefore appear to present a contraindication[9, 10] to the test, since conjugation and excretion in the bile of the phenolphthalein do not occur in a diseased liver and it is consequently excreted in the urine in the free form.

All observers agree that cardiovascular-renal disease and the blood dyscrasias give a high percentage of positive results, probably due to greater mucosal permeability. According to Steigmann and Dyniewicz,[9] in these cases the free phenolphthalein appears when the conjugated form is in a concentration lower than 5 mg. per cent.

It was more difficult to explain the presence of negative tests in patients with gastrointestinal lesions in which a positive test was expected. All persons tested had acute gastrointestinal complaints and all diagnoses were corroborated by laboratory and roentgenologic findings. The patients with gastrointestinal disturbances, all taken from the acute wards, suffered from peptic ulcer, gastric and colonic malignancies, ulcerative colitis, amoebic dysentery, typhoid, acute gastroenteritis, diverticulitis of the colon, intestinal tuberculosis, and hematemesis. It is possible that some of the carcinomas were not ulcerated and allowed no avenue of absorption, or that the other ulcerative lesions developed some protective antiabsorptive process. Rowland[10] suggests that this is due to retained food or an adherent exudate on the ulcerations. Kremer, Shore, and Wiesel[19] are of a similar opinion. It may be that the dosage is the important factor, since 1.5 grains of phenolphthalein may give a negative test repeatedly, while 4 grains gives a positive test regularly in the normal person.

Other objections are those associated with the gastrointestinal disturbances which the test is capable of producing (Table IV). The routine use of the alcoholic solvent (10 c.c. of 95 per cent alcohol) in cases with acute involvement of the upper tract, notably hematemesis and gastric ulcer, is considered inadvisable. It has a tendency to produce temporary gastric distress and enhances the possibility of further bleeding. Clark[11] commented on the fact that alcohol taken upon an empty stomach in concentrations above 10 per cent produces gastric irritation and causes an initial secretion of gastric juice which is followed by an inhibition persisting for as long as twenty-four hours. The resulting increased gastric secretion is, of course, especially inadvisable in peptic ulcer. Other vehicles for the phenolphthalein were found by Fantus and

Dyniewicz,[6] after a comprehensive study of solvents, to be unsuitable because of insolubility or marked irritability, making the use of an alcoholic solvent imperative.

TABLE IV

TOXIC EFFECTS OF THE PHENOLPHTHALEIN TEST

	CASES	PERCENTAGE
Nausea	41	30.0
Vomiting	9	6.5
Abdominal cramps	16	11.7
Diarrhea	11	8.0
Laxative effect	72	47.0
Anorexia	20	13.2
Skin	0	0
Pyrexia	5	3.6
Kidney manifestations	0	0

Many of our patients complained of nausea and a few vomited; the vomiting may have been due in part to a superimposed psychic factor. Indigestion of subsequent food or anorexia was a source of annoyance for another group of patients. The most serious digestive derangement, however, was diarrhea. Although Woldman states that a small dose of 0.1 Gm. is without laxative effect, Fantus and Dyniewicz[7] record these effects in practically all of their normal individuals, even with approximately half as large a dose as that employed in the test (0.06 Gm.). Some degree of laxation occurred in almost half our patients, while others[9] report it in as high as 80 per cent of their patients. Abramowitz[8] has indicated that the laxative action of phenolphthalein is further enhanced when the drug is ingested in an alcoholic solution. Gunn[12] pointed out that after its solution by bile and alkali in the intestine, phenolphthalein produces purgation by mild irritation of the small bowel and especially the large bowel. Some of it may be absorbed and re-excreted into the intestine so that its purgative action may last for a few days. Such was the case in our group of patients, especially those with typhoid, ulcerative colitis, or gastric malignancy. It was even more pronounced where diarrhea existed previously. Loose movements persisted anywhere from one to three days and were accompanied by a sudden rise in temperature, ranging between 101° and 104° F., notably with lesions of the lower bowel. Abdominal cramps lasting several hours also occurred, especially if diarrhea was present. This was undoubtedly due to the irritant effect of both the solvent and solute as previously described. Newman,[13] in enumerating seventeen types of atypical skin lesions, visceral hemorrhages and ulcerative colitis, as well as other evidences of phenolphthalein toxicity, mentioned that even small amounts used for "pink icing on cakes, for coloring of candies, and in pink mouth washes and dentifrices" may cause these lesions.

We did not notice any skin manifestations in any of the cases, although such lesions of the fixed erythematous and multiforme types have been described by Fox,[14] Wise and Abramowitz,[15] Newman,[13] and others. Eruptions due to phenolphthalein, although not very frequent, should be watched for. No evidences of renal irritation or the constant slight albuminuria after small doses of 0.05 to 0.15 Gm., described by Hydrick,[16] were noted. However, this has not been confirmed by Abramowitz,[8] Fantus and Dyniewicz,[17] and others.

In a comprehensive survey of all phenolphthalein reactions reported up to 1935, Abramowitz[8] stated the advisability of first testing a patient for susceptibility to phenolphthalein with a small dose of 0.032 Gm. (0.5 gr.) or even less. The type of idiosyncrasy that exists points toward a sensitization of the patient to the drug, especially in view of the small dosage and the occasionally long intervals between ingestion of the drug and the reactions.

Although von Vamossy[18] raised objections against the existence of numerous reactions which have been accumulated to date, the evidence remains overwhelmingly in favor of their occurrence (except possibly for the presence of albuminuria), making the test further objectionable in numerous cases.

CONCLUSIONS

1. The phenolphthalein test for gastrointestinal lesions, although simple and rapid to perform, is not without many important disadvantages. The error in our series is 24 per cent for controls and 48 per cent for persons with definite gastrointestinal lesions. Other investigations concur in the high percentage of error.

2. Explanations are offered for the marked discrepancies occurring in both false positive and false negative reactions.

3. The test is definitely contraindicated in hematemesis, most cases of acute ulceration, and acute diseases of the colon or acute exacerbations of chronic ulcerative conditions. It is also valueless in cases of jaundice, cardiovascular-renal disease, and blood dyscrasias.

4. Among the toxic gastrointestinal disturbances resulting are nausea, vomiting, laxation, abdominal cramps, diarrhea, temperature elevation and, more rarely, skin manifestations.

5. The phenolphthalein test cannot be recommended as a diagnostic procedure for the differentiation of organic disease from functional disorders of the gastrointestinal tract, or between gastrointestinal disease and diseases outside the digestive system which display symptoms referable to the stomach or intestines.

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LABORATORY METHODS

GENERAL

AN INEXPENSIVE POLARIZING DEVICE FOR MICROSCOPES*

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THE study of urinary sediments of patients with renal disease, especially when massive albuminuria is present, should always include the use of a polarizing microscope to discover the presence of doubly refractile bodies (anisotropic lipoids). Nicol prisms serve excellently to convert a standard into a polarizing microscope at a cost of about $50.00. Recently, polaroid material mounted between white optical glass was introduced as a less expensive substitute for Nicol prisms. The price of this polarizing equipment, from $15.00 to $30.00, may be too costly for many physicians for such specialized microscope accessories. During the past two years we have utilized the relatively inexpensive polaroid film of 3/1,000 inch thickness to convert a standard into a polarizing microscope at a cost of $2.00. In order to enable physicians to study urinary sediments in their own laboratories by polarized light with this inexpensive medium, we are presenting our method of converting the standard laboratory microscope into one which will serve this purpose.

Polaroid film may be purchased† in four inch squares at a cost of $4.00 each (sufficient for two microscopes). Care must be exercised in handling the film to avoid surface scratches. The eyepiece is removed from the microscope, and its outer lens mount is unscrewed. On the outer circular rim on the undersurface of the lens mount, a thin layer of clear, neutral Canada balsam is carefully applied. A circular piece of polaroid film is cut from the four inch square of a diameter just a trifle smaller than the total diameter of the rim, and is carefully applied to the rim over the balsam. After the balsam has hardened, within forty-eight to seventy-two hours, the outer lens mount is screwed into the eyepiece. The latter is replaced into the microscope and will serve as the analyzer. A wider circular piece of film is then carefully cut from the remainder of the four inch square to fit the horizontal slot under the condenser below the stage of the microscope. This larger piece of film is carefully inserted into the slot; it should not be removed thereafter. It will serve as the polarizer.

The converted microscope may be used for the usual laboratory purposes by rotating the eyepiece so that the axes of the analyzer and polarizer are

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†Polaroid film may be purchased from the Polaroid Company, 285 Columbus Avenue, Boston.

parallel. By slow rotation of the eyepiece one produces a reddish-purplish-black field (when the analyzer and the polarizer are at right angles) in which doubly refractile bodies in the urinary sediment will appear as glistening maltese crosses. Care must be exercised to distinguish these bodies from debris, crystals, etc.

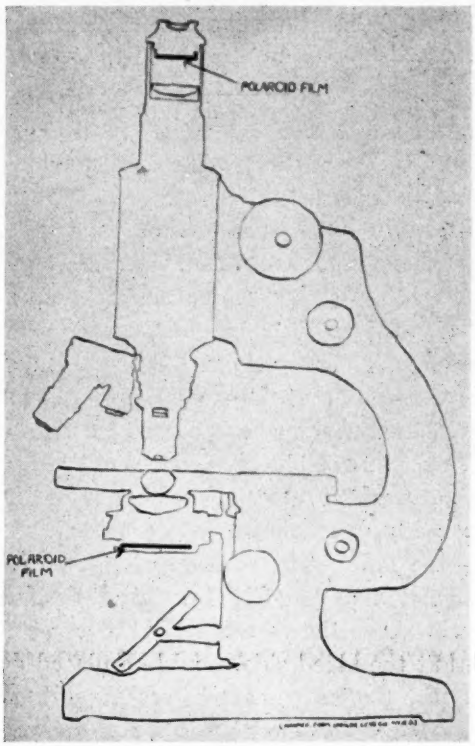

Fig. 1.—Diagram of polarizing microscope showing location of polaroid film.

With the polarizing microscope, prepared according to the method described (Fig. 1), we have found doubly refractile bodies in the urinary sediments of patients with the nephrotic syndrome associated with glomerulonephritis, amyloid disease, thrombosis of the renal veins, diabetes and hypertension.

THE MEASUREMENT OF RED BLOOD CELL DIAMETER BY THE DIFFRACTOMETER*

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THE measurement of red blood cells by the diffraction or halo method was first described by Pijper[1] in 1924. He seems to have used it only in the diagnosis of pernicious anemia, as did Eve.[2] Because it was of simple construction and required little time it was soon to come into wider clinical use.[3-5] However, there is little report of a halo method gaining popularity in this country, and only two of our textbooks[6, 7] consider the procedure in detail.

In view of the fact that so little actual experience has been reported, we would like to present our impressions. Since July, 1936, we have been using a diffractometer manufactured by the Spencer Lens Co. The instrument itself is very easy to use and may be attached to any monocular microscope. There is no optical mechanism involved. A small black cylinder with a pin hole in the bottom is attached in place of the objective. The eyepiece is replaced by a second cylinder with a pinhole in the top and an adjustable device on the sides for measuring the halo. The microscope mirror is adjusted to give the most light from any good lamp (see Fig. 1). The slide is inserted in a slot in the top cylinder and rests above the measurer. Either a stained or an unstained smear may be used, but the blood side should be placed down.

When one looks into the instrument he sees a white spot in the center surrounded by a yellow halo which shades into a red periphery. The two parallel black bands on the outside are adjusted so that they just touch the outer rim of the red halo. The reading is then taken from the sliding scale and compared with a conversion table to give the mean red blood cell diameter in microns.

We have done approximately a thousand determinations by this method. Every patient having even a mild degree of anemia has at least one mean cell diameter, more often he has several. Very high white counts interfere with a distinct halo; for this reason, we do not attempt readings in patients with leucemia. Occasionally polycythemia offers technical difficulties and an extreme degree of anisocytosis may blur the outline of the halo.

The success of securing an accurate reading lies in having a smear of one cell thickness. The good smears give a clear-cut halo, and it is easy to determine the exact edge of the red circle. Herein lies one of the main sources of error, for two observers may not agree on the outline and thus may obtain different readings. Table I shows how these results may vary between two people. The maximum variation between the readings of two observers using the same smear is 0.4 μ, as compared with 0.3 μ when the same observer used different slides. Even this slight error makes very little diagnostic difference.

*From the Presbyterian Hospital, New York.
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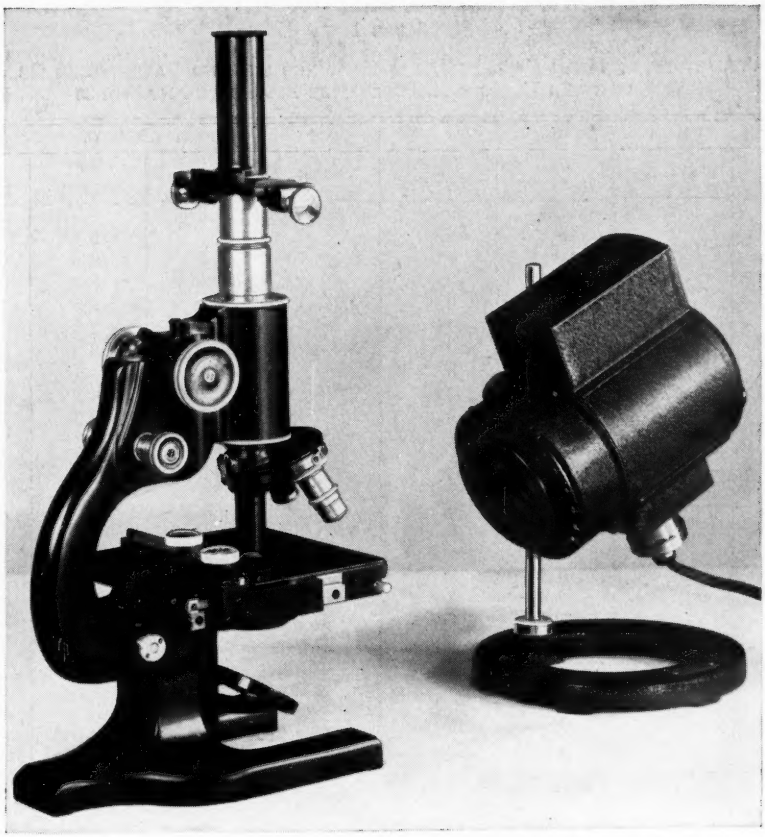

Fig. 1.—The diffractometer. (Courtesy of Spencer Lens Co.)

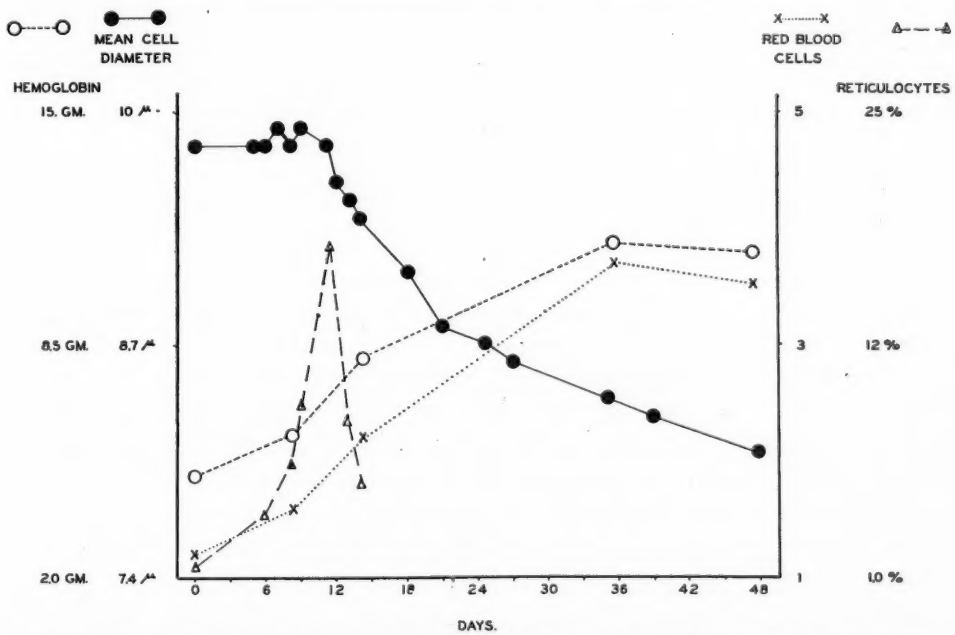

Fig. 2.—Showing the mean cell diameter, hemoglobin, red blood cell, and reticulocyte response to liver extract in a patient with pernicious anemia.

TABLE I

SHOWING VARIATIONS IN MEAN CELL DIAMETER READINGS ON TWO PATIENTS AS OBTAINED BY TWO OBSERVERS USING THE SAME SLIDE AND DIFFERENT SLIDES

PATIENT M. M.				PATIENT A. C.			
DATE	SLIDE	1ST OBSERVER	2ND OBSERVER	DATE	SLIDE	1ST OBSERVER	2ND OBSERVER
2/17/39	1	9.6	9.6	3/23/39	1	8.4	8.3
2/18/39	1	8.8	9.2		2	8.4	8.4
	2	9.0	9.3	3/24/39	1	8.6	8.4
	3	8.8	9.0		2	8.4	8.4
	4	8.9	9.1	3/25/39	1	8.3	8.4
2/20/39	1	9.1	9.3		2	8.4	8.4
	2	9.2	9.4	3/28/39	1	8.3	8.5
2/21/39	1	8.9	9.1		2	8.4	8.3
	2	9.1	9.0	3/29/39	1	8.3	8.4
2/23/39	1	9.2	8.9		2	8.5	8.5
	2	9.3	9.0	3/30/39	1	8.7	8.5
2/28/39	1	8.9	8.8		2	8.6	8.5
	2	9.1	8.9	3/31/39	1	8.6	8.4
3/ 3/39	1	8.9	8.9		2	8.7	8.4
	2	8.9	8.9	4/ 3/39	1	8.4	8.3
3/ 4/39	1	8.8	8.9		2	8.5	8.4
	2	8.8	8.8	4/ 4/39	1	8.5	8.5
3/ 7/39	1	8.7	8.9		2	8.5	8.5
	2	8.7	8.7	4/ 5/39	1	8.5	8.4
3/ 8/39	1	9.9	8.8		2	8.4	8.5
	2	9.0	8.8	4/ 6/39	1	8.6	8.5
3/ 9/39	1	8.9	8.8		2	8.4	8.5
	2	8.8	8.8	4/ 7/39	1	8.4	8.4
3/13/39	1	8.9	9.0		2	8.5	8.3
	2	8.8	8.8				

TABLE II

MEAN RED BLOOD CELL DIAMETER RESULTS OBTAINED BY THE DIFFRACTOMETER METHOD COMPARED WITH THE PRICE-JONES TECHNIQUE

PATIENT	PRICE-JONES METHOD		DIFFRACTOMETER METHOD	
	1ST OBSERVER	2ND OBSERVER	1ST OBSERVER	2ND OBSERVER
1	7.0	7.8	6.9	7.0
2	7.5	7.9	7.6	7.7
3	7.2	8.1	7.2	7.3
4	7.5	7.8	7.5	7.5
5	7.3	7.9	7.2	7.2
6	7.7	8.1	7.5	7.6
7	8.5	9.0	8.9	9.1
8	7.7	8.5	7.9	7.7
9	6.9	8.0	7.6	7.4
10	7.8	8.7	7.5	7.5

As a matter of interest two of us obtained mean cell diameters on the same smears by the Price-Jones and Diffractometer methods. Table II shows a difference of as much as 1.1 μ with the Price-Jones method, and only 0.2 μ with the diffractometer method. Needless to say, there is a vast difference in the time consumed by the two procedures, the halo method requiring only a minute or two, and the direct measuring thirty minutes or more.

The halo method may be criticized because it does not measure cell thickness. However, there are few cases of anemia in which mean corpuscular volume is more helpful in determining microcytosis or macrocytosis than mean cell diam-

eter. Technical errors may limit the accuracy of both methods proportionately. A smear preparation is much easier to obtain from the standpoint of both the patient and technician.

No claim of perfection is made for the diffractometer, but over a period of three years it has proved itself a simple means of obtaining red blood cell size within a reasonable range of error. It is an instrument which might be a real help in any hematologic laboratory.

CONCLUSIONS

1. In over 1,000 mean cell diameter determinations the diffractometer has been found to be simple, efficient, and sufficiently accurate for all clinical purposes.

2. Tables are presented to show that the errors inherent in the method are relatively insignificant.

3. The more general use of this method is suggested.

I am indebted to Miss Illyne for her technical assistance.

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AN ECONOMICAL AND EFFICIENT DISTILLING DUST TRAP

LOIS M. BRAGG, A.M.T., DENISON, TEXAS

PRACTICALLY no laboratory worker has failed at one time or another to experience the need for an efficient means of keeping dust from entering the distillate in routine laboratory distillations.

For a number of months I have used one of the ordinary spiral waxed paper drinking cups, such as is usually found in almost every hospital or business institution. These cups are conical in shape, and in use the tip is simply cut off and the cup pushed up on the condenser tube with the large end down. In this manner the cup may be slipped down so that it covers the mouth of the receiving flask, thus providing a protective shield against dust.

A CONTRAST STAINING METHOD FOR HEMOSIDERIN PIGMENT IN HEART-FAILURE CELLS

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THE methods in use to demonstate hemosiderin pigment offer no means of differentiating the heart-failure cells from the surrounding leucocytes. In some cases it is difficult to demonstrate these cells because no contrast staining is offered. Isolation of such cells is also difficult because the entire smear is colorless save for the stained hemosiderin pigment granules which themselves appear in unstained cells.

A new staining method here presented allows for a contrast. In the sputum or urine, or other material for examination, leucocytes appearing with heart-failure cells are counterstained by Wright's stain, and the hemosiderin takes a bright green stain. The hemosiderin pigment stands out in sharply contrasted granules against the well-stained basic blue and acidophilic red of the stained leucocytes and other cells.

Method for Staining.—(1) Make smear of sputum in usual way. (2) Air dry. (3) Fix with heat. (4) Apply a 10 per cent solution of potassium ferrocyanide and an equal quantity of tenth-normal hydrochloric acid to the slide. Flood the slide thoroughly. (5) Allow this to stand for fifteen minutes. (6) Wash in water. (7) Dry thoroughly. (8) Apply Wright's stain. (9) Add buffered water for five minutes. (10) Wash in water. (11) Dry thoroughly and examine under oil.

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A NEW MODEL MOUSE CAGE

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A NEW type of cage provides optimum living conditions for animals, which are indispensable requisites for trustworthy experimental work in biological laboratories. Fig. 1 shows the cage and run-way ready for use except nesting material. Fig. 2 shows the nest and run-way disconnected. The length over-all is 36 inches; the run-way is 30 by 4 by 3 inches.

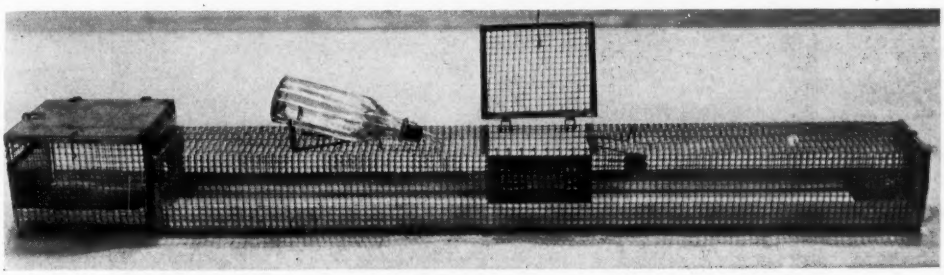

Fig. 1.—*A*, False bottom for nesting cage; *B*, nesting cage, 5¼ by 5¼ by 4 inches, holds 5 or 6 adult mice; *C*, lid giving access to run-way; *D*, false bottom—aluminum 30 by 3¾ inches, ½ inch curb; *E*, wire fastener and door—aluminum—for closing end of run-way; *F*, salad holder which prevents contamination of fresh vegetables and fruits.

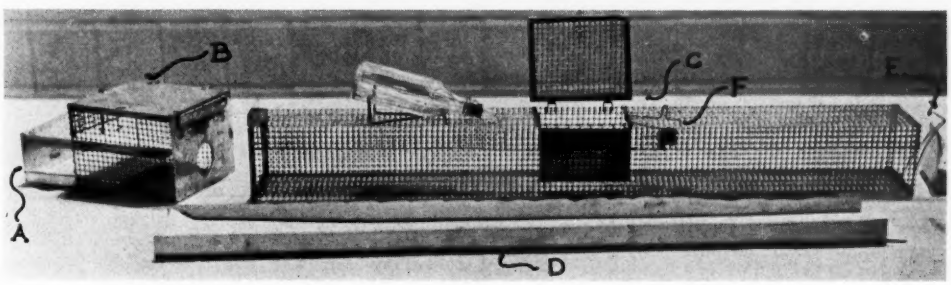

Fig. 2.

The black space on the side of the run-way is the feed box. The water bottle is supported by a wire cradle. The nest is supported securely and easily on the run-way by a metal strip pointing downward toward the circular opening, which engages with a vertical strip on the top of the run-way. Removal and replacement of the nest are thus made easy.

A thick mat of paper pulp—heavy blotting paper—covers the false bottom of the run-way. It is to be replaced by a fresh one as soon as it becomes soiled. This is accomplished quickly and easily by opening *E*, withdrawing the soiled mat and inserting a clean one. The mice prudently deposit their excreta at *E*, as far away from the nest as possible.

To economize laboratory floor space, the cages are supported on a rack which holds sixteen units. Forty-eight cages on three racks, housing 250 mice, would require only a small corner of a room, 4 by 11 feet.

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The square boxcage, shown on the floor of Fig. 3, is a standard type of cage used extensively in biological laboratories. Ventilation, sanitation and opportunity for exercise are utterly inadequate. The long run-way of the new

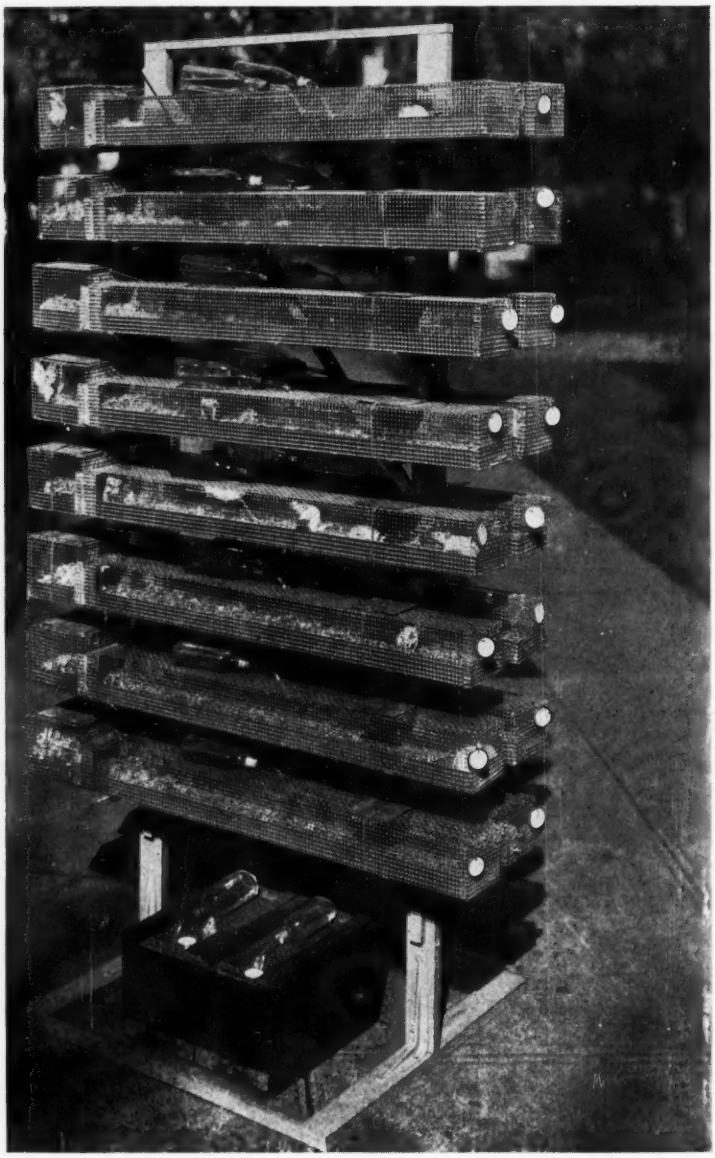

Fig. 3.

model gives an abundance of fresh air and unlimited chance for exercise. Note the mouse walking upside down on the ceiling of the fifth cage on the rack. The hygienic advantages of the modern cage over the old standard type are glaringly obvious. This kind of cage is admirably adapted for high school biology classes on account of the minimum amount of time required to keep the unit tidy and odorless, simply by replacement of the floor mat once daily.

A SIMPLE AND ACCURATE MICROFRAGILITY TEST FOR MEASURING ERYTHROCYTE RESISTANCE*

A Combination Microhemopipette Method

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NUMEROUS methods have been devised and revised for the purpose of testing the resistance of the red blood cells to various hemolytic and hemotoxic agents. Quantitation of hemolysis may be expressed in terms of any of the following criteria: (1) the depth of red color due to dissolved hemoglobin developing in the hemolytic media in which the erythrocytes have been suspended; (2) the percentage volume of the unhemolyzed corpuscles, as determined by the hematocrit method after centrifugation, since the hypotonic solutions cause the cells to expand until the maximum distensibility (the "critical" volume) has been obtained; or (3) the number of intact erythrocytes remaining in the hemolytic solutions, as enumerated by the hemocytometer method. These criteria may be used singly or in combination.

Each of the above-mentioned methods of measurement possesses its own merit and yields satisfactory results in the hands of experienced observers who have mastered its technical details. The simplest and perhaps the most reliable criterion, however, appears to be the one which estimates the degree of hemolysis by the tinctorial density of the red supernatant fluid resulting from the presence of varying amounts of hemoglobin liberated from ruptured corpuscles. This is the oldest principle of the test as first devised by Hamburger (1883) and adapted for clinical use by von Limbeck (1896) and by Ribierre (1903). Because of its simplicity and accuracy, this method, with repeated modifications by Theobald Smith (1904), Karsner and Pearce (1912), Hill (1915), Giffin and Sanford (1919), Fontaine (1921), Daland and Worthley (1935), and Beebe and Hanley (1936), is still today most extensively used.

The hematocrit method, first thoroughly investigated by Hamburger (1902) and simplified by van Allen (1925) and his followers, particularly Silvette (1927) and Guest and Wing (1939), is unsatisfactory unless accompanied by other criteria, since the estimation of packed cell volume often yields doubtful results, primarily because of the presence of "ghost" cells and of unavoidable errors arising from differences in the speed and duration of centrifugation. This is particularly true when determinations are to be made on patients with severe anemia accompanied by oligocythemia.

Enumeration of intact erythrocytes remaining in the hypotonic solutions of varying concentrations is also an old method, first proposed by Chanel

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(1880) and later refined by Simmel (1923), Leake and Pratt (1925), Waugh and Chase (1928), and Whitby and Hynes (1935). This method may be accurate, but the presence of hemolyzed cells with intact stroma among the truly unhemolyzed cells may lead to confusing results. Moreover, the use of a counting chamber for each concentraton of salt solutions is time-consuming, and the preparation of hypotonic solutions, as devised by Simmel, is complicated. To overcome this defect, Harvey (1937) proposed the use of only one pipette and a standard hypotonic diluent of 0.35 per cent solution of sodium chloride.

A variety of hemolytic and hemotoxic substances have been employed to induce hemolysis for testing the erythrocyte resistance. The agent longest in use is sodium chloride in different concentrations, this having been first utilized by Johannes Duncan in 1867 and by Malassez in 1872, and in 1900 Vaquez pronounced a scale consisting of gradually increasing dilutions to be the "ideal method." Other substances used for the purpose are sodium oxalate, sodium sulfate, saponin, and sapotoxin, as well as other vegetable and animal poisons. The stock solution recommended by Simmel contains sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium biphosphate, and sodium bicarbonate. Another method, employed by Theobald Smith (1904), and by Wiseman and Bierbaum (1932) is that of using diluted serum or plasma obtained from the blood samples to be tested for erythrocyte fragility. The Grancher's serum used by Chanel contained one gram of sodium sulfate in 40 c.c. of serum.

The hemolytic action of these various substances upon erythrocytes differs according to physical and chemical characteristics. Certain chemicals (benzene and indol) cause the cell membrane to become more permeable, while others (sugar and lecithin) increase the resistance of erythrocyte stroma. The presence in the blood serum of inhibitory or accelerative substances is also an important factor in understanding the mechanism of hemolysis in vitro as well as in vivo.

Nearly all the methods mentioned, except the hematocrit method, require the use of venous blood, a feature which offers a decided obstacle in testing the fragility of red blood cells in small infants. The method herein described is an attempt to reduce the technique of erythrocyte fragility test to its simplest terms, by using only six different concentrations of hypotonic sodium chloride solutions and the least possible amount of capillary blood. Incidentally, it must be emphasized that the micromethod here described, because of the exactness required in mixing the saline with distilled water in correct proportions, yields results that are far more accurate than any of the prevailing macroscopic methods.

DESCRIPTION OF THE MICROFRAGILITY TEST

Instruments and Utensils Required for the Test.—For the performance of the microfragility test a set of six combination microhemopipettes* with metal holders (Kato, 1938) are used. The hypotonic solutions are prepared from a 0.9 per cent solution of chemically pure recently dried sodium chloride in distilled water, the dilutions being made according to the proportions recommended

*Combination microhemopipettes and metal holders are manufactured by the Fisher Scientific Co., Pittsburgh, Pa.

in Table I. For obtaining the blood sample (see below) a hanging-drop slide with a central well (14 mm. in diameter and 3 mm. in depth) is employed.* For mixing the blood with hypotonic solutions a white porcelain spot plate provided with twelve depressions is most useful, since the entire series of dilutions can be prepared in rapid succession.

TABLE I

GRADUATED SCALE OF CONCENTRATIONS OF SODIUM CHLORIDE SOLUTION RECOMMENDED FOR USE IN MICROFRAGILITY TEST

PIPETTE NO.	NaCl (0.9%) (C.MM.)	DISTILLED WATER (C.MM.)	OXALATED BLOOD (C.MM.)	SALT CONC. (C) (%)	NORMAL HEMOLYSIS SPAN
	30	18	12	0.630	
	29	19	12	0.615	
	28	20	12	0.600	Goat
	27	21	12	0.585	
	26	22	12	0.570	
	25	23	12	0.555	
	24	24	12	0.540	
	23	25	12	0.525	
	22	26	12	0.510	
	21	27	12	0.495	Rabbit
	20	28	12	0.480	
	19	29	12	0.465	
1	18*	30	12	0.450	
2	17*	31	12	0.435	
3	16*	32	12	0.420	
4	15*	33	12	0.405	
	14	34	12	0.390	
	13	35	12	0.375	Man
5	12*	36	12	0.360	
	11	37	12	0.345	
6	10*	38	12	0.330	
	9	39	12	0.315	
	8	40	12	0.300	
	7	41	12	0.285	
	6	42	12	0.270	
	5	43	12	0.255	

*Indicates the most important concentrations which must be included in the test.

The total amount of hypotonic salt solution in all the concentrations tabulated above is 60 c.mm., allowing 10 c.mm. for loss incident to manipulation.

The salt concentration (C) of any mixture may be calculated from the equation:

$$\frac{0.9\%\ \text{NaCl (c.mm.)} + \text{Blood (c.mm.)}}{60} \times 0.9 = \text{C}.$$

Obtaining Blood Samples.—Capillary blood is obtained from a deep skin puncture in the usual manner and is brought immediately into contact with either dry heparin powder or double oxalate mixture, the latter of which will enable the observer to use the same sample for testing the prothrombin clotting time if indicated (Kato, 1940). The proportion of either anticoagulant to the amount of blood sample should be in the neighborhood of 1:500. In this proportion these anticoagulants exert a negligible influence upon the osmotic equilibrium of the blood cells and produce no alterations on the packed cell volume. Thus, for the performance of microfragility test approximately 0.2 c.c. of capillary blood is received into the well of a hanging-drop slide, containing 0.4 mg.

*Hanging-drop slides (culture slides) can be obtained from Clay-Adams Co., New York, N. Y.

of an anticoagulant. This amount of anticoagulant can be accurately delivered by introducing 20 c.mm. of a 2 per cent solution. The double oxalate solution has the following composition:

Potassium oxalate	0.75 Gm.
Ammonium oxalate	1.25 Gm.
Distilled water	100.00 c.c.

The heparin solution of similar concentration is made from crude heparin in distilled water.

The blood sample prepared according to the foregoing method is then placed in a moist chamber until the test is performed.

Preparation of Erythrocyte Suspensions.—To prepare the suspensions of erythrocytes in hypotonic solutions, the specified amount of 0.9 per cent stock solution of sodium chloride is first drawn up into a combination microhemopipette and carefully expelled into one of the depressions of a spot plate. Using the same pipette, the distilled water, also in the corresponding specified amount, is measured out and added to the saline. The two reagents are then gently but thoroughly mixed by alternately drawing up and expelling the mixture in the bore of the pipette. Finally, with the same pipette the specified amount of well-homogenized blood is thoroughly mixed with the hypotonic solution, and the resulting suspension is at once drawn up to the top mark (50 c.mm.) of the pipette, thus avoiding any waste of the suspension. The total amount of suspension, prepared according to the proportions recommended in Table I, is exactly 60 c.mm., allowing for loss of approximately 10 c.mm., which usually adhere to the surface of the utensil during the preparation of the mixture. This manipulation requires a little skill which, however, can be mastered easily after a few trials. If the blowing force is too strong, bubble formation in the mixture will invariably cause a shortage of the suspension to fill the pipette to the top mark. If too much of the suspension is lost in this way, or in any other way, a new one must be prepared to insure uniform results, especially with reference to the cell volume in each pipette.

After the suspension is drawn up to the top mark, the two ends of the pipette are at once closed by immobilization in the metal holder, and correctly labeled according to the number corresponding to each dilution. The same process is repeated for each concentration of salt solutions. After the entire series of dilutions have been prepared, they are left in horizontal positions on a flat surface and allowed to remain so for at least one hour, occasionally reversing the positions of the pipettes to permit the complete hemolytic action of solutions upon the suspended erythrocytes. The pipettes in their metal holders are then centrifuged for ten to fifteen minutes at 2,500 r.p.m. The supernatant fluid in each pipette and the cellular layer at its bottom may now be observed as to the extent of hemolysis.

Recording the Results.—The concentrations recommended for the series of six pipettes used in the microfragility test have been selected to include only the two critical ranges; namely, the range in which initial hemolysis normally occurs (first four pipettes) and the range in which complete hemolysis is most frequently observed (last two pipettes). For normal human blood beyond in-

fancy the concentration at which initial hemolysis occurs is usually 0.435 per cent (the second pipette), and occasionally 0.420 per cent (the third pipette), and complete hemolysis is 0.330 per cent concentration (the last pipette). These two critical concentrations can be used to designate the span of hemolysis, conveniently recorded as 0.435/0.330. Undoubtedly, even under normal conditions slight variations may occur, but the selection of the two critical ranges recommended is based upon experience gained in performing a large number of tests on normal human blood. If too great a deviation is found in either direction, a repetition of the test is necessary by changing the scale of concentrations in whichever direction indicated.

TABLE II

AVERAGE NORMAL FRAGILITY OF HUMAN ERYTHROCYTES

(Microfragility Test)

PIPETTE NO.	SALT CONC. (%)	DEGREE OF HEMOLYSIS	VOLUME OF GHOST CELLS (C.MM.)	VOLUME OF INTACT CELLS* (C.MM.)
1	0.450	0	0	5.0
2	0.435	±	0.1	5.5
3	0.420	+	0.5	6.0
4	0.405	++	1.0	5.0
5	0.345	+++	Indistinct	2.0
6	0.330	++++	Invisible	0

*The figures in this column represent the values of packed cell volume of unhemolyzed erythrocytes in actual number of cubic millimeters after centrifugation (compare with Fig. 1). Since these values depend upon the percentage volume of packed erythrocytes in undiluted whole blood, the volume in each diluted specimen may be more accurately expressed in relative percentage of the packed whole cell volume.

In recording the degree of hemolysis the depth of the red color, due to the presence of dissolved hemoglobin, affords the most reliable criterion, but the packed erythrocyte column found at the bottom of the pipette also yields valuable information (Table II). In the second pipette, in which initial hemolysis normally occurs, the red color of the supernatant fluid may be so slight that its detection may require a careful comparison with the first pipette in which hemolysis is negative. But a trace of hemolysis in the second pipette is almost invariably accompanied by the presence of a very thin layer of transparent hemolyzed cells, the so-called "ghost" cells, overlying the packed intact erythrocytes. As the concentration of the salt solution decreases, the "ghost" cell layer will be seen to increase in proportion to the augmenting intensity of the red color in the supernatant fluid. At the same time there is a gradual decrease in volume of opaque intact erythrocytes after having increased at initial hemolysis, until complete hemolysis is reached, at which point there is no demonstrable column of intact cells and the red color of the solution is so intense that the "ghost" cells are completely obscured (Fig. 1). The percentage volume of opaque unhemolyzed erythrocytes in each pipette after centrifugation may also be recorded in comparison with the packed cell volume of the whole blood, but owing to certain unavoidable errors in dealing with a small amount of blood such as recommended for the microfragility test here described, the observation of the cell volume alone does not yield consistent results of any particular value.

Attempts at exact quantitation of hemolysis by use of a colorimeter employing standard solution of hemoglobin for comparison have been made by

some observers, particularly Lapicque and Vast (1899), Lepeschkin (1932), and Waugh and Asherman (1938), but such procedures appear to be entirely unnecessary inasmuch as the quantitative estimation of liberated hemoglobin is not the primary object of the test. For practical clinical purposes it is quite sufficient to indicate the range of hemolysis by simply noting the concentrations of salt solution in which hemolysis begins and ends, in the manner indicated in Table II.

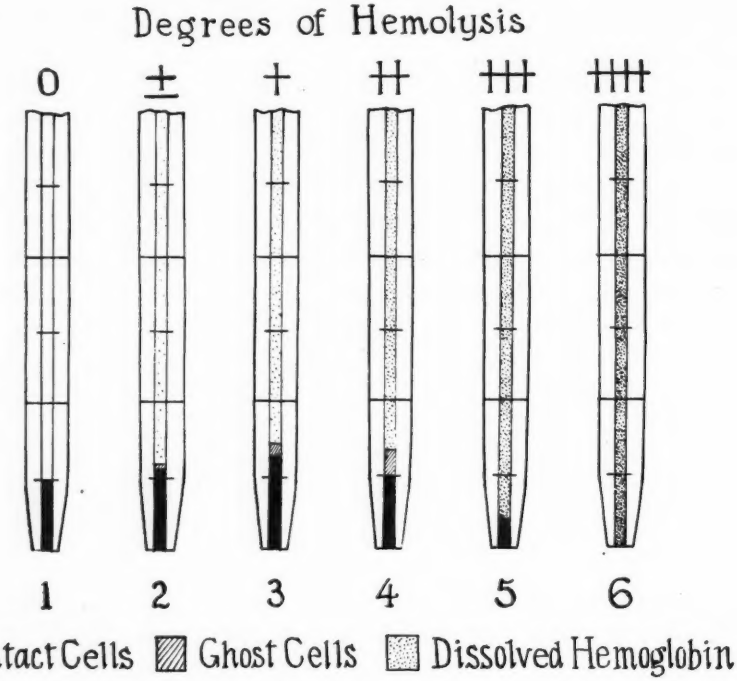

Fig. 1.—Six combination microhemopipettes (1-6) used in the microfragility test. Note the absence of hemolysis in the first pipette and complete hemolysis in the last pipette. The maximum cell volume is obtained in pipette 3 and the initial hemolysis is seen in pipette 2.

COMMENT

The microfragility test here described is an attempt to simplify the most commonly used method for measuring the resistance of erythrocytes to hypotonic solutions of sodium chloride. Three distinct advantages of this test are: (1) simplicity in technical manipulation; (2) small amount of blood required for the test; and (3) accuracy attained by the use of graduated microhemopipettes which facilitate the exact observation not only of the degree of hemolysis in the supernatant fluid, but also of the volume of hemolyzed cells which overlies the column of opaque intact erythrocytes. The microfragility test may be most conveniently used in pediatric practice and in hematologic research on small experimental animals.

According to the results obtained from observations on a number of normal human beings, the microfragility test reveals the average initial hemolysis that occurs at the sodium chloride concentration of 0.435 per cent and the com-

plete hemolysis at 0.330 per cent, giving an average of 0.1 per cent as a normal hemolysis span. Slight variations may be found from time to time, but under normal conditions the resistance of human erythrocytes against hypotonic salt solutions remains remarkably constant. Any significant deviation from the normal range mentioned, especially in the point of initial hemolysis, usually indicates the presence of erythrocyte anomaly and calls for repeated observations. A few examples of instances in which abnormal fragility has been found are summarized in Table III.

TABLE III

ABNORMAL FRAGILITY IN VARIOUS CONDITIONS

DISEASE	R.B.C. (MILLIONS)	HB. (GM. %)	PACKED CELL VOLUME (%)	CORPUSCLE VOLUME (cμ)	ICTERIC INDEX (MURPHY UNITS)	INITIAL HEMOLYSIS (% CONC. NaCl)	FINAL HEMOLYSIS (% CONC. NaCl)
Congenital hemolytic icterus (Remission)	3.75	10.2	30	88	30	0.450	0.375
Catarrhal jaundice	4.82	12.9	37	85	120	0.435	0.255
Leucemia, acute	1.56	4.4	18	125	2	0.405	0.315
Anemia, post-hemorrhagic	3.54	6.7	22	69	2	0.405	0.330
Rheumatic carditis	4.33	13.8	40	103	2	0.450	0.360
Anemia, chronic infection	1.81	2.8	12	74	2	0.390	0.285
Hemorrhagic disease of newborn	6.21	18.9	62	100	15	0.480	0.345
Hemorrhagic disease of newborn	6.10	19.0	63	103	30	0.510	0.300
Normal newborn	5.09	16.0	55	108	20	0.525	0.330

One of the most important clinical conditions in which the test proves to be most useful is in jaundice so frequently seen in various types of liver disease and blood dyscrasias. Since Chauffard in 1907 first demonstrated the increased fragility of erythrocytes in congenital hemolytic jaundice, numerous observers have confirmed this striking phenomenon. In one instance of this disease the microfragility test performed during a remission disclosed only a slight increase in erythrocyte fragility, namely, 0.450/0.375 per cent, indicating a definite shortening of the span of hemolysis. In a case of moderately severe catarrhal jaundice with pronounced bilirubinemia (van den Bergh 15 units) the initial hemolysis occurred at a normal concentration (0.435 per cent), but the hemolysis was not complete until the concentration reached an abnormally low level (0.255 per cent), indicative of a great prolongation of the hemolysis span. During the terminal stage of a case of acute leucemia with a marked anemia (erythrocyte count 1,560,000) and extreme leucopenia (leucocyte count 900), there was a slight increase in erythrocyte resistance (0.405/0.315 per cent). Practically identical results in fragility tests were obtained in a case of moderately severe posthemorrhagic anemia (erythrocyte count 3,540,000) in a 7-year-old child. In a case of rheumatic carditis in a quiescent stage erythrocyte fragility was slightly decreased (0.450/0.360 per cent), while in one instance of advanced rheumatoid arthritis with a pronounced anemia (erythrocyte count

1,810,000) an abnormal increase in erythrocyte resistance (0.390/0.285 per cent) was noted. In normal newly born infants it is customary to find a distinct increase in erythrocyte fragility (average 0.510/0.300), a phenomenon which satisfactorily explains the pathogenesis of physiologic jaundice of the newborn.

The most remarkable instance of greatly increased erythrocyte fragility was seen in a newborn infant with erythroblastosis fetalis (icterus gravis neonatorum) with a high icteric index (150 Murphy units). The microfragility test performed toward the end of the first twenty-four hours of life revealed

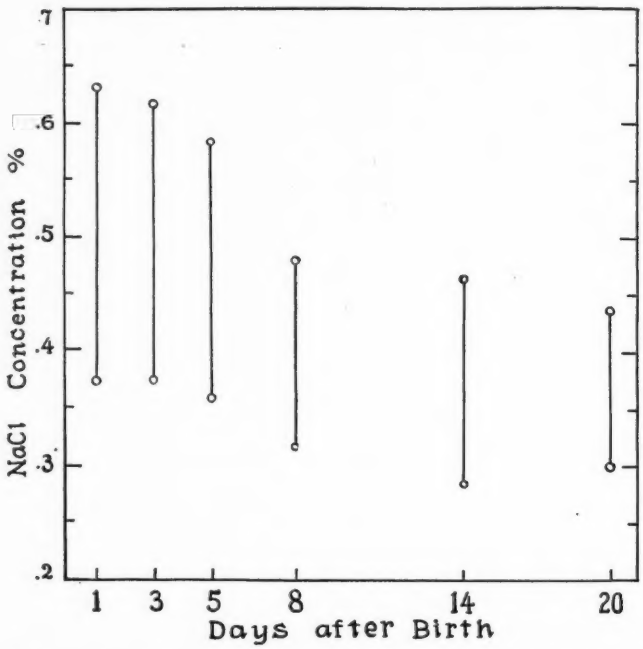

Fig. 2.—Hemolysis spans of erythrocytes in a case of erythroblastosis fetalis (icterus gravis neonatorum). Note increased fragility with prolonged hemolysis spans during the first five days of life and gradual return to the normal span upon recovery.

initial hemolysis at a concentration of 0.630 per cent and complete hemolysis at 0.375 per cent (Fig. 2). Upon repeating the test on subsequent dates, it was found that erythrocyte resistance gradually increased so that by the fourteenth day of life the cells began to hemolyze at a concentration slightly above the limits of normal range. Incidentally, it is to be noted that the gradual decrease in erythrocyte fragility in this infant coincided with the decrease in the percentage number of nucleated red blood cells, which on the first day of life was as high as approximately 75 per cent of the total nucleated blood cells.

These findings, though purely accidental, throw some light on the pathogenesis of extreme jaundice so characteristic of this disease. Icterus neonatorum, both physiologic and pathologic, has been the subject of repeated investigations by numerous observers, but as yet no conclusive evidence of its underlying etiology has been demonstrated. The accidental findings here recorded suggest very strongly the possibility of increased erythrocyte fragility, with consequent

bilirubinemia as being the fundamental cause of jaundice in the newborn. Moreover, in all probability, this increase in fragility is due to the rapid destruction of nucleated red blood cells which are so abundantly found in various organs as well as in the peripheral blood. The severity of jaundice appears to be in direct proportion to the increase in erythrocyte fragility, since erythrocyte fragility in normal newly born infants with physiologic type of icterus is only slightly increased and never to the extent seen in icterus gravis neonatorum. A more complete investigation of erythrocyte fragility in the newborn will be given in a later report.

FRAGILITY OF MAMMALIAN AND AVIAN ERYTHROCYTES

The fact that the resistance of erythrocytes against hemolytic agents is specific not only in each species of animals, but also in different individuals of the same species, was conclusively demonstrated by Hamburger (1902). The results of observations obtained by means of the microfragility test performed on the blood of mammals and birds, summarized in Table IV, reveal a great variation in erythrocyte fragility in the different species.

TABLE IV

MICROFRAGILITY TEST IN MAMMALS, PIGEON, AND MAN

SPECIES	AVERAGE NORMAL R.B.C. (MILLIONS PER C.MM.)	PACKED CELL VOLUME (%)	CORPUSCLE VOLUME (cμ)	INITIAL HEMOLYSIS (% CONC. Nacl)	FINAL HEMOLYSIS (% CONC. Nacl)
Goat	16.00	55	34.5	0.615	0.480
Sheep	7.50	33	44.0	0.600	0.450
Rat	7.00	50	71.4	0.480	0.375
Rabbit	5.00	40	72.7	0.570	0.450
Mouse	10.00	80	80.0	0.535	0.330
Dog	6.50	55	84.6	0.450	0.360
Guinea pig	5.50	50	91.0	0.450	0.330
Pigeon	2.75	33	120.0	0.465	0.360
Man (infant)	6.00	55	90.0	0.510-0.450	0.315-0.300
Man (child)	4.00	35	88.8	0.435	0.330
Man (adult)	5.00	45	90.0	0.420	0.330

Data on the fragility tests in animals demonstrate the definite relationship existing between the volume as well as the shape of the red blood cells and their degree of resistance against hypotonic salt solutions. The fact that the fragility parallels the diameter of the cells was first noted by Vallery-Radot and Lehritier (1919), while Castle and Daland (1937) proved that differences in the susceptibility of various types of erythrocytes to hemolysis are due principally to differences in form and not to differences in osmotic behavior. Haden (1939), in numerous and painstaking studies of the peculiarities of erythrocytes in congenital hemolytic icterus, concludes that spherocytosis, which is so characteristic of this disease, is the essential factor in increased fragility.

In this connection it is also of interest to note the striking observation recorded by Rywosch (1907), namely, that the hemolytic action of hypotonic saline upon the resistance of mammalian erythrocytes exhibits a reverse of their resistance to saponin. Ponder, Saslow, and Yeager (1930), in re-examining a large number of resistance series of erythrocytes from various animals, have

proposed the hypothesis that in any hemolytic system certain physicochemical changes take place which affect the permeability of cell membranes, specific to the erythrocytes of each species and giving rise to different resistance series.

The increased fragility of erythrocytes in pigeons, in spite of their high mean corpuscular volume, requires separate consideration, inasmuch as their red blood cells are all nucleated.

SUMMARY

A new microfragility test, utilizing the same principle as that originally employed by Hamburger and used for clinical purposes by von Limbeck and by Ribierre, and later modified by numerous workers, particularly Giffin and Sanford, but greatly simplified and especially adapted for use in small infants and animals, is described.

As tested by this method, initial hemolysis of normal human erythrocytes is found to occur at an average of 0.420 to 0.435 per cent concentration of sodium chloride solution, while complete hemolysis is most frequently seen at 0.315 to 0.330 per cent concentration. The normal range or span of hemolysis is thus approximately 0.1 per cent concentration. The simplicity and accuracy of the test are the features especially adapted to routine hematologic examination.

A few illustrative examples of the results obtained by this method in various diseases characterized by abnormalities in erythrocyte fragility are reported. The striking increase in erythrocyte fragility in a case of erythroblastosis fetalis (icterus gravis neonatorum), as revealed by the microfragility test, suggests the fundamental mechanism in the causation of icterus in the newborn.

The normal hemolysis ranges of erythrocytes in a few mammals and pigeons, as measured by the microfragility test, are also recorded in this preliminary report.

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CHEMICAL

THE USE OF OSTEOTROPIC DYES IN A MODIFIED LINE TEST FOR VITAMIN D*

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THERE are three methods for the estimation of vitamin D: the estimation of the ash content of bones (humerus or femur);[1] the line test;[2] and the x-ray examination of bones.[3] The determination of ash content has never received wide acceptance, as it is a much more tedious process than the line test. Hume and co-workers[4] evaluated this technique and found that when the percentage of ash was plotted against the logarithms of the doses, a linear relationship between the two variables was revealed, showing that as the doses were increased in geometrical progression the calcification increments advanced in arithmetical series. It is an excellent method but, for practical reasons, it is difficult to apply.

The x-ray technique was studied by Bourdillon and associates,[5] who attributed such an increased accuracy to the method that they listed twelve different degrees of healing in rachitic rats. Compared to the bone ash method, radiographic comparison is equally accurate, and because it is easier to apply it is the better of the two methods.

The line test is the more generally accepted procedure because it is inexpensive, requires no special apparatus, and presents no technical difficulties. Bills and others[6] in a "critique" of the line test, state that by using 100 animals the error is reduced to 4 per cent. The line test depends upon the conversion of silver phosphate to black colloidal silver following exposure to light. Bills lists the different types of healing as the epiphyseal, in which new calcium is laid down in a narrow line at the distal edge of the cartilage; the metaphyseal, in which deposition occurs at the metaphysis; and the diaphyseal, in which the first calcification is diaphyseal and contiguous with the trabeculae that remain after rickets develops. The latter is useless for the line test. The line test is most delicate, since it detects very early stages of healing which x-ray will not show. Bone, whether newly deposited or not, gives the reactions involved in the silver method. Deposition of bone when it does occur is not necessarily along the line of calcification examined in the line test. These facts frequently complicate the accurate diagnosis of a bone section.

As uncalcified tissue will not stain with hematoxylin, and calcified tissue will, a method of hematoxylin staining has been used, but, again, there is no differentiation between the newly formed and the old bone.

Gottlieb[7] suggested the use of alizarin and purpurin in the staining of newly formed bone. The suggestion was made that the mechanism involved the local

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concentration of calcium ions. The use of alizarin and of purpurin was studied by Cameron,[8] who injected sodium alizarin sulfonate in a 2 per cent solution. Bones started developing the color in five minutes and became most intensely colored in twelve hours. Anthrapurpurin and purpurin gave less pronounced results. Cameron used the method in studying fracture callus in the rat. The callus was unstained at ten days, deeply stained from ten to sixty days, and practically unstained thereafter. Alizarin was also used to stain the calcified areas produced by hypervitaminosis D. A method used by Brash[9] consists of feeding madder and then stopping the administration so that new bone growth is white, while all bone formed during the madder feeding period is stained from pink to red. This seemed to be the clue to a highly specific line test modification. The mechanism of the osteotropic dye is suggested by Ercoli[10] to be an adsorption phenomenon. The sodium alizarin sulfonate is irreversibly fixed and a red complex is formed. Phosphate ion is liberated in this mechanism.

EXPERIMENTAL

Sodium alizarin sulfonate, a water-soluble derivative of alizarin, is recommended by Cameron[8] for its osteotropism. We administered 1 c.c. of a 2 per cent solution to 60 Gm. rats and found we were above or at the fatal dose. The dosage was cut to 0.2 c.c. of 2 per cent sodium alizarin sulfonate and found to be harmless, but it gave no distinct coloration to the bones. The compound showed no marked osteotropism, probably because of its water solubility.

Next, a 2 per cent suspension of alizarin in water was used. One cubic centimeter each was injected intraperitoneally into 20 rats. No bone coloration was observed in any of the supplemented animals. Here the complete insolubility of the alizarin prevented its being effective. Examination of the peritoneal cavities of these animals showed unabsorbed dye in large amount.

Finally, sodium alizarinate was used in a 1 per cent solution in water at a pH of 8.0. In one experiment of twenty-four-hour duration, 1 c.c. of the 1 per cent sodium alizarinate solution was injected intraperitoneally. At the same time, supplements containing varying amounts of vitamin D in oil were given orally. Twenty-four hours later the animals were killed, and the radii and ulnae were removed, split lengthwise, and examined microscopically. The negative controls showed no coloration or a faint pink tinge, indicating the absence of new bone formation. If any coloration is seen, it is generally disseminated, coloring the entire bone and assuming no definite pattern, a state such as occurs following new calcium deposition. Three rats receiving a total of 0.3 units of vitamin D showed 1+ and 2+ healing by our scale. The calcium deposition may occur along the line of calcification in the epiphysis or the diaphysis, and yet distinct coloration, which can be used in assigning a value of healing, is observed. Three rats receiving a total of 1 unit of vitamin D showed a 2+ and 3+ healing. As the bones are semitransparent, it is possible to observe macroscopic differences in color intensity without cutting them. It is possible that a twenty-four-hour test can be developed, using macroscopic examination.

Ten rats were used in a two-day test. One cubic centimeter of 1 per cent sodium alizarinate was given intraperitoneally on the first day to all rats. Six-

tenths unit of vitamin D was given to the supplemented animals in two doses of 0.3 unit each. Forty-eight hours after the first dose of vitamin D was given, the animals were killed and the bones were examined. Values of 3+ and 4+ were assigned to the degree of healing seen.

Arbitrary values are assigned to divide into six divisions, from 0 to 6, the range of healing from absence of coloration with no vitamin D to the coloration of the bone at complete healing. A value of 6 indicates complete healing. Chart 1 illustrates these stages of healing.

TABLE I

COMPARISON OF SILVER AND ALIZARIN LINE TESTS

Two-Day Test

(Numbers indicate the degree of healing.)

NEGATIVE CONTROLS		STANDARD 0.95 UNIT DAILY		STANDARD 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	0	2	3	3	5
0	0	2	3	3	5
0	0	1	2	2	5
0	0	2	3	0	4
0	0	3	3	2	5
1	0	3	3	3	4
0	0	1	2		
UNKNOWN 0.1 C.C. CALCULATED 0.31 UNIT DAILY		UNKNOWN 0.3 C.C. CALCULATED 0.93 UNIT DAILY		UNKNOWN 0.6 C.C. CALCULATED 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	1	2-3	3	4-5	5
0-1	1	0-1	4	4-5	5
1	1	2	3	3-4	5
0	1	4	4	4	5
0-1	1	0	3	2	4
0	1	2	3	5	5
1	1	2	3	2	4

A series of tests was conducted, using both the silver staining[2] and the alizarin techniques. The results are recorded in Table I. This table shows a more uniform result where alizarin was used. In this group the sodium alizarinate was given intraperitoneally on the date of the first dosage with vitamin D, and the bones were examined forty-eight hours later.

The second series of sodium alizarinate animals was given intraperitoneal dye (1 c.c. of 1 per cent) after the third dosage of vitamin D in a series of five daily doses. The bones were removed and examined on the sixth day. An improved coloration was noted which, compared to the silver line test, gave the results in Table II.

A third series for comparison was conducted by giving the sodium alizarinate (0.3 c.c. of 1 per cent) on three different occasions: at the time of the first vitamin D supplement, on the third day, and when the final dose (fifth day) of vitamin D was given. The group showed no material improvement over the second series in accuracy, and the additional administrations are, therefore, regarded as unnecessary. The results of this smaller group are recorded in Table III.

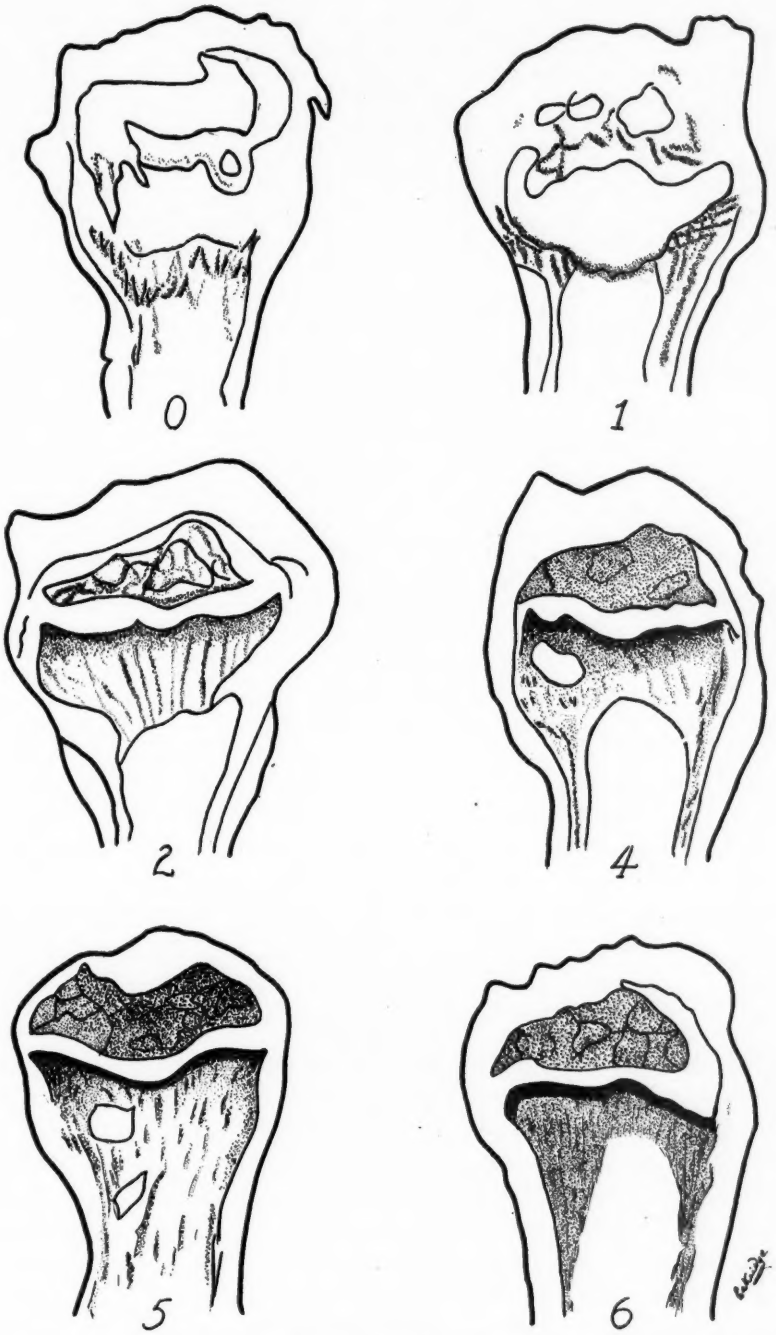

Chart 1.—Alizarin line test. Numbers indicate the degree of healing. Number 6 indicates complete healing.

TABLE II

COMPARISON OF SILVER AND ALIZARIN LINE TESTS

Five-Day Test

(Numbers indicate the degree of healing.)

NEGATIVE CONTROLS		STANDARD 0.95 UNIT DAILY		STANDARD 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	0	3	5	4-5	6
0	0	2	5	5	6
0	0	1	4-5	3	6
1	0	3	4	5	6
0	0	0	5	4	6
1	0	3	4	3	5
UNKNOWN 0.1 C.C. CALCULATED 0.31 UNIT DAILY		UNKNOWN 0.3 C.C. CALCULATED 0.93 UNIT DAILY		UNKNOWN 0.6 C.C. CALCULATED 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	1-2	3-4	4-5	4-5	6
1-2	2	2	5	5	6
0-1	2	3	5	3-4	6
1-2	3	2	4-5	5	6
2	2	4	4	5	6
1-2	2	1	4	3-4	5

TABLE III

COMPARISON OF SILVER AND ALIZARIN LINE TESTS

Five-Day Test, Alizarin Injected for Three Days

(Numbers indicate degree of healing.)

NEGATIVE CONTROLS		STANDARD 0.95 UNIT DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN
0	0	3	5
1	0	3-4	4-5
0	1	2-3	5
1-2	0	3	4-5
1-2	0	4	5
0	0	3	5
1-2	0	2	5
1	1	3	4-5

DISCUSSION

Cameron[8] has pointed out that alizarin is a nearly specific stain for calcium. It reacts readily in vitro or in vivo with recently deposited calcium salts, either normal or pathologically formed, but it fails to stain older deposits. Silver nitrate is not a test for calcium or phosphate; the black reaction is given by a variety of solids, the anion being more important than the cation. As pointed out by Bills and associates,[6] deposition of calcium following administration of vitamin D may not occur along epiphyseal or diaphyseal lines, and, if it does not, the silver line test is useless. Hematoxylin does not stain calcium salts, though it often identifies areas in which changes favorable to the deposition of calcium salts are taking place. Alizarin stains only newly formed bone. It stains these calcium deposits whether they are epiphyseal, diaphyseal, or metaphyseal. It follows that greater specificity, greater applicability, and greater speed result from the use of alizarin.

SUMMARY

A modified line test involving the use of an osteotropic dye, alizarin, is introduced. Greater specificity, greater applicability, and greater speed result from its use.

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RECOVERY OF CARBON TETRACHLORIDE FROM TISSUE*

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THE difficulty encountered by Gettler and Siegel[1] in the recovery of pure carbon tetrachloride after addition to tissue seemed to me to be due to the heating of the carbon tetrachloride in the presence of tissue. The first step in their method is steam distillation. While they were able to recover the carbon tetrachloride by rectification after steam distillation of carbon tetrachloride in water, the method failed when applied to carbon tetrachloride added to tissue. In the latter case a part of the carbon tetrachloride was converted to chloroform. To obviate heating, the method here described was devised.

THE APPARATUS

The apparatus is shown in Fig. 1. The ground glass joints connecting *A* and *B*, and *B* and *C* are 45-50. The tube *D* is a Gettler collection tube[1, 2] sealed into the bottom of *C*. This collection tube is 13 cm. long, has a diameter of 0.7 cm., and a capacity of 3 c.c. graduated in twentieths. By means of rubber vacuum tubing *E* is connected to two glass traps in series. The second trap is connected through an ordinary closed-end mercury manometer to a vacuum pump.

Flask *A* is supported by a condenser clamp at the neck, and *C* is prevented from falling by copper wire fastened tightly around the neck of *C* and coiled loosely about the horizontal portion of *B*. The usual glass hooks sealed in above and below the glass joint and rubber bands may also be used.

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PROCEDURE

The distillation flask *A* was immersed to the bottom of the neck in dry ice and acetone contained in a large Dewar flask. Some glass wool had been placed in the Dewar to prevent spontaneous overflowing. Half of the tissue, ground lamb's brain, was introduced into *A*. The temperature of the carbon tetrachloride was taken (room temperature). It was introduced by means of a pipette graduated in hundredths. The second half of the tissue was then introduced. After the ground glass joints had been greased with Lubriseal, *B* and *C* were attached, and the stopcock in the side arm of *B* was closed, *E* was connected to the first trap, and the tissue and carbon tetrachloride were allowed to freeze for the length of time indicated in Table I.

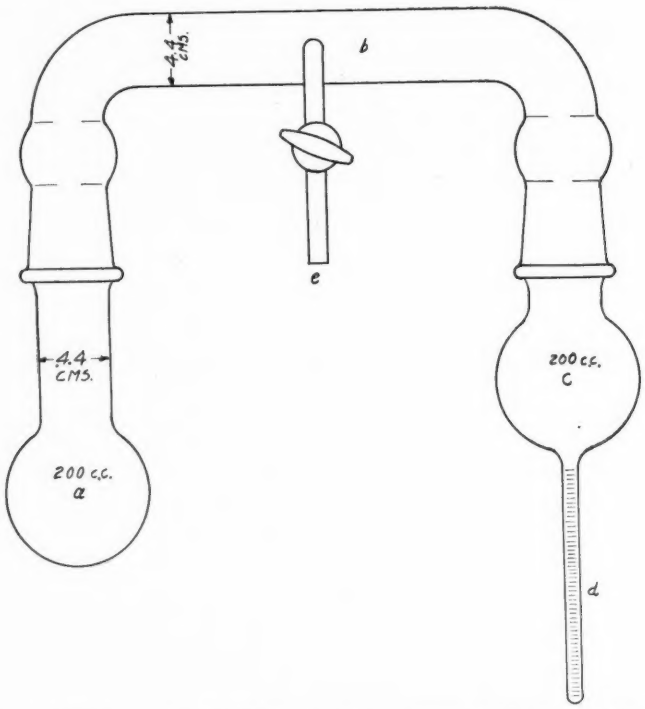

Fig. 1.—Apparatus for distillation and measurement of recovered liquid.

Each of the two traps was immersed in a Dewar containing dry ice, acetone, and glass wool; the stopcock in the side arm of *B* was opened and the vacuum pump was turned on. The pump was allowed to run for about ten minutes after the minimum pressure was attained, which averaged about 0.5 mm. The stopcock was closed and the rubber tubing was disconnected.

The large Dewar containing dry ice and acetone was shifted from *A* to *C*, the level of the liquid being adjusted to the midpoint of the bulb *C*. The flask *A* was immersed to the bottom of the neck in water at about 25° C., and the distillation was allowed to run for the length of time indicated in Table I. At the end of the distillation the stopcock was opened to allow the pressure inside to come to that of the atmosphere, and then closed. The apparatus was then raised so that only the lower half of the collection tube *D* was immersed in the dry ice

and acetone. When all the solid in *C* had melted, *C* was disconnected at the ground glass joint, stoppered with a cork, and the collection tube immersed in water and kept at the temperature at which the carbon tetrachloride had been measured until no change in volume could be observed. The amount of the distilled water above the carbon tetrachloride varied from 0.05 to 2 c.c. In spite of scrupulous cleaning, first with soap and water and then with chromic acid or concentrated nitric acid and drying in an oven, the bulb *C* always contained droplets of moisture at the end of the melting. After water was dropped into *C* to wash this moisture into *D*, additional carbon tetrachloride was sometimes recovered.

TABLE I

NO.	TISSUE (GM.)	CCl_4 (C.C.)	FREEZING TIME (MIN.)	DISTILLATION TIME (MIN.)	RECOVERY (C.C.)	RECOVERY %	BOILING POINT ORIGINAL	BOILING POINT RECOVERED MATERIAL	REFRACTIVE INDEX ORIGINAL	REFRACTIVE INDEX RECOVERED MATERIAL
1	75	1.00	120	90	0.95	95.0	76.6	76.6	1.4607	1.4603
2	50	1.00	60	135	0.95	95.0	76.6	76.6		1.4602
3	50	1.00	45	60	0.93	93.0	76.7	76.4		1.4607
4	50	1.00	120	100	0.90	90.0	76.6	76.6		1.4602
5	50	1.00	60	155	0.93	93.0	76.6	76.6		1.4603
6	50	1.00	130	45	0.95	95.0	76.6	76.6		1.4603
7	45	1.00	50	60	0.93	93.0	76.4	76.4		1.4607
8	50	1.00	80	80	0.95	95.0	76.6	76.6		1.4605
9	50	0.50	65	82	0.40	80.0	76.6	76.5		1.4603
10	50	0.50	60	60	0.40	80.0		76.3		1.4607
11	50	0.75	110	90	0.70	93.3	76.6	76.7		1.4610
12	50	0.70	60	60	0.63	90.0	76.6	76.7		1.4608
13	50	0.70	75	60	0.65	92.9	76.6	76.6		1.4606
14	100	1.00	135	155	0.95	95.0	76.6	76.6		1.4610
15	100	1.00	60	120	0.95	95.0	76.6	76.6		1.4609
16	100	0.70	60	115	0.64	91.4	76.6	76.5		1.4609
17	100	0.70	65	140	0.61	87.1	76.7	76.2		1.4606
18	100	0.50	60	105	0.45	90.0	76.7	76.7		1.4605
19	100	0.50	80	90	0.45	90.0	76.6	76.8		1.4607

The recovery in cubic centimeters and percentage is given in Table I. The purity was determined by the microboiling point, Siwoloboff's method,[3] and the refractive index of the carbon tetrachloride layer. The boiling point of the original carbon tetrachloride was determined at as near the same pressure as was practicable. The refractive indices were corrected for temperature by applying the general rule: refractive index decreases 0.0004 per degree Centigrade rise above 20°.

All recovery samples and the original were tested for chloroform in carbon tetrachloride by the cyclohexanol, alpha-naphthol, potassium hydroxide method.[4] Each gave a negative result.

A small amount of frost always appeared in the glass traps. The rate of evaporation on warming to room temperature suggested water. On only one occasion was there a sufficiently large drop for a boiling point determination. In this case it was water.

Putrefaction of the tissue apparently does not affect the purity of the distillate. In determinations 1, 4, and 17 in Table I, the tissue was very putrid, and in many other cases it was several weeks old.

DISCUSSION

I believe some carbon tetrachloride is lost during its introduction into the distilling flask. There is always a strong odor of carbon tetrachloride during this process. Also, while calibration of the collection tube against the pipette using mercury shows a check to 0.01 c.c., the transfer of 1 c.c. of carbon tetrachloride from the pipette to the collection tube at room temperature shows a loss of 0.025 c.c. It may be possible that the carbon tetrachloride present in tissue poisoned in vivo may be frozen at a low temperature, the tissue ground and introduced before thawing sets in, and the carbon tetrachloride recovered with less loss. It is also possible that some carbon tetrachloride is vaporized during the melting, or that some remains in the distillation flask.

The method should be adaptable for use in isolating ether or any other liquid immiscible with water which boils below 100° C. from tissue or other similar mixtures.

SUMMARY

A low temperature, low pressure distillation method for the recovery of carbon tetrachloride after addition to tissue is described.

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I wish to acknowledge with appreciation the very valuable suggestions made by Dr. Joseph Greenspan of the Chemistry Department, Brooklyn College.

A SIMPLIFIED MICRO TEST OF PLASMA PROTHROMBIN*

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A SIMPLIFIED micro test of plasma prothrombin based on the principles of the Smith test has been found to be as accurate as the Smith test used by us for the determination of the prothrombin clotting activity of the blood. A useful micro Quick test has been devised by Kato[1] but it is as complicated as the Quick method[2] because of the number of steps and ingredients involved in the test. However, as in the Smith method,[3] the simplified micro test as herein suggested depends upon the use of only blood and thromboplastin. Utilizing the principle of the Smith test, the micro test was investigated and was found to be surprisingly accurate and simple to perform. The test was done on patients suspected of having a hypoprothrombinemia and checked by the Smith method. The results were recorded in per cent of prothrombin clotting activity (Tables I and II).

The micro test is performed in the following manner: A puncture wound is made in the ear, finger, or heel, and with a Kato microhemopipette 30 to 40 c.mm. of blood are drawn into the tube. This is transferred immediately to a watch glass and mixed with 10 c.mm. of thromboplastin.† The glass is tilted back and forth until a clot is formed. The time recorded for the formation of the clot is divided into the control prothrombin clotting time obtained in the same manner from a normal individual. The result is the per cent of prothrombin clotting activity of the patient. Dangerous levels are those below 70 per cent. Hemorrhage may occur when these levels are reached. The time found by this method is usually five to eight seconds longer than the time by the Smith test. However, the percentage result is as accurate as in the Smith method. The only difficulty encountered in the micro test is the determination of the end point of the clot. With practice the exact end point can be readily determined.

The advantages of the micro method are obvious. It does not require a venipuncture and necessitates very little equipment and time to perform. Since venipuncture is unnecessary, it may be done on infants without difficulty. It

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†Thromboplastin is obtained from rabbit brains or lungs. Approximately 25 Gm. of the material is macerated in a mortar and mixed with about 100 c.c. of physiologic saline solution. This is allowed to stand in the icebox for three hours. The supernatant fluid is decanted and contains the active thromboplastin. The solution is kept in the icebox and is warmed to room temperature before using. If the prothrombin clotting time for a normal individual is less than twenty seconds, the thromboplastin solution should be diluted with saline so that the normal prothrombin time will be between twenty and thirty seconds. The activity of the thromboplastin decreases daily; thus a prothrombin clotting time for the sample of thromboplastin used must be made on a normal individual at the time the test is performed on the patient.

TABLE I

COMPARATIVE PLASMA PROTHROMBIN VALUES FOR THE SMITH AND THE MICRO METHOD TAKEN UPON NORMAL INDIVIDUALS USING THE SAME THROMBOPLASTIN SOLUTION

Approximately 5 to 8 seconds difference occurs between the times of the two methods, but the percentage result is accurate for each method.

PATIENT	PROTHROMBIN CLOTTING ACTIVITY	
	SMITH METHOD	MICRO METHOD
1 Normal	26 sec. or 100%	33 sec. or 100%
2 Normal	26 sec. or 100%	34 sec. or 100%
3 Normal	28 sec. or 100%	34 sec. or 100%
4 Normal	30 sec. or 100%	35 sec. or 100%
5 Normal	31 sec. or 100%	37 sec. or 100%
6 Normal	30 sec. or 100%	37 sec. or 100%
7 Normal	30 sec. or 100%	36 sec. or 100%

TABLE II

COMPARATIVE PLASMA PROTHROMBIN VALUES IN 8 PATIENTS SUSPECTED OF HAVING A HYPOPROTHROMBINEMIA

The per cent plasma prothrombin activity as determined by the micro method is found to coincide with the clotting activity as determined simultaneously by the Smith method.

PATIENT AND DIAGNOSIS	PROTHROMBIN CLOTTING ACTIVITY	
	SMITH METHOD	MICRO METHOD
1 Choledocholithiasis	43 sec. or 72%	50 sec. or 70%
2 Choledocholithiasis	38 sec. or 71%	46 sec. or 74%
3 Choledocholithiasis	36 sec. or 84%	40 sec. or 80%
4 Choledocholithiasis	30 sec. or 100%	36 sec. or 100%
5 Cholecystitis	30 sec. or 88%	38 sec. or 88%
6 Cholecystitis	25 sec. or 84%	30 sec. or 83%
7 Carcinoma of pancreas	32 sec. or 79%	38 sec. or 80%
8 Cholelithiasis	24 sec. or 86%	32 sec. or 87%

may be done as quickly as a leucocyte or erythrocyte count, and in accuracy compares favorably with the Smith test.

The microhemopipette of Kato may be purchased from the Fisher Scientific Co., Pittsburgh, Pa.

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CONSISTENT BLOOD SUGAR VALUES OBTAINED FROM RABBIT HEART BLOOD SAMPLES*

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THE use of the rabbit for blood sugar estimations has often been questioned. Still the animal has been used, and in most cases has furnished the required information even if the data obtained were handled conservatively. Certain objections have remained, nevertheless, and in particular the influence of excitement. Even the trained animal becomes excited during ear vein puncture for peripheral blood; and in taking heart blood under ordinary precautions, excitement nearly always occurs and internal injury frequently takes place. In order to overcome these and other well-known objections, the following technique was put into operation, and the normal blood sugar values obtained (Hagedorn-Jensen[1]) were subjected to statistical analysis.

TECHNIQUE

No anesthetic or assistance is needed in carrying out the technique, but a quiet room and a rabbit board of adequate length are essential for success.

The rabbit is gently lifted by its ears onto the lower short edge of the board and is held in an upright position (Fig. 1) until loops are placed on its extremities. After its hind legs are tied close, the rabbit is slowly lowered until its back rests on the board (Fig. 2). The forelegs are slowly drawn in the direction of the head and tied. Tying is necessary to keep the animal from rolling over. These steps are facilitated if the grip on the rabbit's ears is maintained with one hand until the other has completed the tying. The animal may be kept quiet for hours if its nose is stroked at intervals, or when deemed necessary.

Heart blood should be taken only after the rabbit has been resting on its back a few minutes. A syringe with a No. 19 or 20 gauge needle has been found best. The needle is introduced from the left costal region, and the blood is withdrawn slowly. When samples are taken at widely separated intervals, the rabbit may be lifted from the board and placed in a box until needed again, without removing the ties from the extremities.

EXPERIMENTS AND RESULTS

Blood sugar estimations were made on samples procured at the following intervals: 15, 30, 60, 120, 180, and 240 minutes. Two series of experiments were done. In one series, including 25 animals, the rabbit was kept on the board in the quiet state for the entire period of four hours. In the other series, 75 animals, the rabbit was placed in a box between the intervals. The latter experiments permitted study of the effect change in position would have on blood sugar values.

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Table I includes the mean values (m) and their respective standard deviation (σm) for the experiments during which the animal was kept supine for four hours, as well as when it was placed in a box between the time intervals. For the six intervals the mean values range between 74.12 and 77.00 mg. per cent in the first series, and between 77.70 and 81.15 mg. per cent in the second.

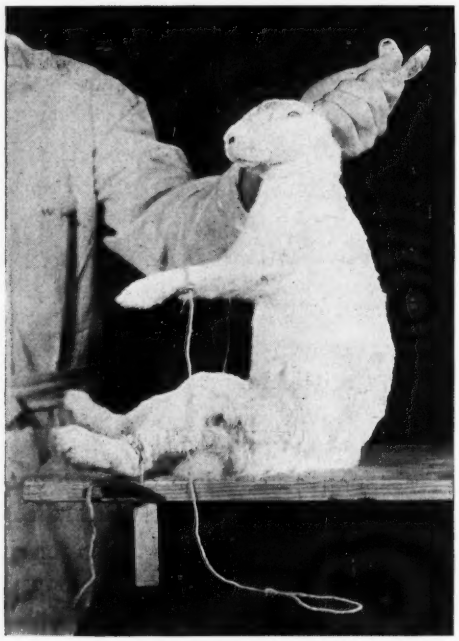

Fig. 1.

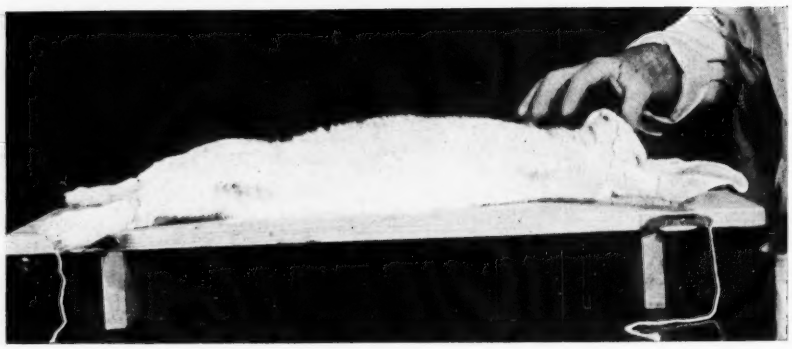

Fig. 2.

The greatest obtained difference, therefore, is 2.88 and 3.45, respectively. The mean of the six means of the former group is 75.45, and of the latter, 79.36, and their difference is 3.89.

DISCUSSION

Statistical study of the blood sugar values revealed fair agreement. The means obtained in the series of experiments when the animal was kept supine for four hours gave obtained differences which fell well within the range of error of the blood sugar method. This was also true when one treated similarly

the means obtained in the series when the animal was placed in a box between the intervals. Comparison of the two series indicated that one gains very little, if anything, by keeping the animal supine for a period of four hours.

TABLE I

BLOOD SUGAR VALUES IN MILLIGRAMS PER CENT OBTAINED BY THE HAGEDORN-JENSEN METHOD

INTERVALS IN MINUTES	25 RABBITS SUPINE FOR 4 HOURS		75 RABBITS NOT SUPINE BETWEEN INTERVALS	
	M	σM	M	σM
15	74.12	±4.80	77.70	±6.20
30	75.00	5.17	78.30	5.60
60	77.00	7.18	79.80	7.63
120	74.67	3.75	81.15	5.60
180	75.50	4.20	79.70	5.83
240	76.40	5.87	79.50	6.24

Several advantages are observed in the technique described. Skill entirely eliminates excitement in the animal. The method of handling, omission of an anesthetic, the facility with which heart blood may be taken in adequate quantities, all help furnish more accurate blood sugar values. With care the animal may be used again and again.

CONCLUSIONS

A technique is described which permits heart blood to be taken from rabbits without the need of an anesthetic or assistance and which renders consistent blood sugar values.

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EFFECT OF INGESTED IRON ON TESTS FOR OCCULT BLOOD IN STOOLS*

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IT IS not infrequently important to determine whether a patient, who is receiving iron by mouth to correct an anemia due to gastrointestinal hemorrhage, is still losing detectable amounts of blood. Tarry stools are not significant while he is ingesting iron. Inquiries, therefore, were instituted among a number of pathologists and laboratory workers to determine whether the usual chemical tests for occult blood in the stools were reliable under these conditions. The diversity of opinions suggested that actual experiment was indicated.

Since it has been found that large amounts of red meat in the diet may give a falsely positive benzidine test for occult blood in the stools, even though the guaiac test usually remains negative, seven persons were first put on a meat-free diet. After several days their stools were tested and found to give no

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reaction for occult blood with either benzidine or guaiac reagents.* Each individual was maintained on the same diet and given in addition 12 grains daily of ferrous sulfate by mouth. As soon as the stools became tarry the benzidine and guaiac tests were again applied. To our surprise the guaiac tests were repeatedly positive and the benzidine tests were consistently negative so long as the ferrous sulfate resulted in tarry stools. The iron was then discontinued and the stools were again tested for occult blood as soon as the tarry characteristics disappeared. Both benzidine and guaiac reagents once more gave negative results as before the iron was administered.

Nine other persons who were found to have both benzidine and guaiac-negative stools on the ordinary hospital diet were given daily 30 grains of ferrous sulfate by mouth. As soon as their stools became black they were found to give a positive reaction with the guaiac test but not with the benzidine test. After discontinuing the ferrous sulfate the stools of these patients gradually became guaiac negative, with the exception of one, as they lost their tarry appearance. This patient continued to produce stools that gave a positive reaction as late as five days after the tarry color had disappeared. The nine patients who had received double the usual therapeutic dose of ferrous sulfate did not produce guaiac-negative stools until several days after the excreta had lost their tarry color, whereas those who received only 12 grains daily gave guaiac-negative stools as soon as the stools regained their normal color.

As a check on these observations the following in vitro experiments were carried out. A solution of ferrous sulfate was made up from the tablets in therapeutic use, and the usual tests were carried out with both benzidine and guaiac reagents. Positive tests were again given by guaiac but not by benzidine. Our pathologist, Dr. F. D. Jones, suggested that the false positives with the guaiac reagent were due to tannin which gave rise to blue iron tannate. Accordingly, the benzidine reagent was used on a solution of ferrous sulfate and ferric chloride to which a small amount of tannic acid powder had been added. A characteristically blue reaction was then obtained as in the case of the guaiac reagent.

Because chlorophyll, which imparts the green color to many vegetables, is composed of pyrrole rings as is the hemoglobin molecule, it was thought that this substance might give rise to false tests for occult blood in the stools. Chlorophyll was, therefore, extracted with alcohol from various green plants and tested with both the guaiac and benzidine reagents. Both tests were negative.

CONCLUSIONS

Benzidine may give a falsely positive test for occult blood in the stools if the patient is eating considerable meat. This appears to be less likely with the guaiac test, which gives a falsely positive reaction for blood if the patient is receiving enough iron by mouth to produce tarry stools. The benzidine test is not affected by iron either in vivo or in vitro. Chlorophyll failed to give a positive reaction with either guaiac or benzidine in vitro.

*Benzidine test: Acidify a bit of feces with a few drops of glacial acetic acid and sprinkle benzidine powder mixed with sodium perborate over the resultant paste. The presence of a blue color indicates a positive reaction.

Guaiac test: Make the feces into a thick paste with water and acidify with a few drops of glacial acetic acid. To this add an equal volume of fresh alcoholic solution of gum guaiac. Mix and add an equal amount of hydrogen peroxide. A blue color indicates a positive reaction.

THE DETERMINATION OF HIPPURIC ACID IN URINE*

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THE estimation of hippuric acid in urine after the ingestion of benzoic acid was recommended by Quick[1] as a test for liver function. According to Weichselbaum and Probstein,[2] Quick's method proved to be unsatisfactory because of an incorrect solubility factor. They modified the method by saturating the urine with sodium chloride before precipitating it with acid and in this manner they not only diminished this solubility factor, but also caused it to be more constant. Employing this modification in our own laboratory, we were unable to obtain satisfactory results. We observed that different urines behaved differently and that the addition of more acid to the filtrates occasionally brought down a further precipitate. This suggested a critical evaluation of the optimum acidity for the quantitative precipitation of hippuric acid added to urine.

In both the original and the modified methods, it was apparent that the importance of pH was not sufficiently stressed. Thus, in the original publication Quick[1] states: "Acidify (acid to Congo red) with concentrated hydrochloric acid—about one c.c., stir vigorously." Weichselbaum and Probstein[2] (page 637) state: "Add 1.2 c.c. of approximately 10 N sulfuric acid and scratch the sides of the flask with a rod to enhance crystallization, etc." Quick[3] in a later publication (page 717) states: "Concentrated hydrochloric acid is added to make urine distinctly acid to Congo red or thymol blue—usually 1 c.c. of the acid is sufficient, but it is absolutely necessary to check with an indicator paper," and adds, "an excess of acid is permissible." It would be more exact if the latter statement were to read: *an excess of acid is imperative.* The following preliminary experiment bears out this point:

To 600 c.c. of normal urine acidified with acetic acid and brought to a boil to remove coagulable material, hippuric acid was added and the solution was filtered. Into each of four beakers 150 c.c. of this urine were measured. Forty-five grams of sodium chloride were added to each, then the procedure, as directed by Weichselbaum, was continued, varying the amount of 10 N sulfuric acid as follows:

	AMOUNT OF 10 N H_2SO_4 ADDED (C.C.)	AMOUNT OF HIPPURIC ACID RECOVERED (GM.)	(%)
1. Acid added as directed by Quick	1.0	1.20	70.6
2. Acid added as directed by Weichselbaum with very vigorous stirring	1.2	1.45	85.3
3. Same as 1; acid added until a precipitate formed and 0.5 c.c. excess	1.5	1.67	98.3
4. Same as 3 but twice the amount of acid was added	3.0	1.70	100.0

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These results show that the acidity used in beakers 1 and 2, as recommended by the previous authors, although definitely acid to Congo red and to thymol blue, is insufficient to precipitate the hippuric acid quantitatively. In beaker 4, where a large excess of acid was used, the precipitate did not redissolve.

TABLE I*

BEAKER NO.	HIPPURIC ACID (GM.)	SLIGHTLY ACIDIFIED URINE (C.C.)	10 N H_2SO_4 ADDED (C.C.)	RECOVERY OF HIPPURIC ACID (GM.)	(DUPLICATE)	% ERROR
1	0.5	100	3.2	0.505	0.51	+3.0
2	1.0	100	2.5	0.990	0.992	−0.1
3	1.5	100	1.2	1.510	1.470	−1.3
4	2.0	100	0.8	1.980	1.950	−2.0
5	3.5	100	0.0	2.640†	2.670†	−24.0†
			2.5	0.806	0.770	−1.7

*The solubility correction given by Weichselbaum was used.

†Precipitate formed on cooling to 20° C. without the addition of acid. To mother liquor 2.5 c.c. acid was added and an additional precipitate was obtained.

The next experiment was planned to simulate clinical conditions and to show the quantitative relationship between the amount of hippuric acid in urine and the amount of inorganic acid required to precipitate it quantitatively. Obviously, the extent of impairment of liver function for which the hippuric acid test is used clinically will vary with different patients, and consequently a varied amount of hippuric acid will be excreted. Since the normal output in a four-hour specimen is given as 3 to 3.5 Gm., it is reasonable to expect that clinically an excretion of from 0.0 to 3.5 Gm. or more can be expected. A normal slightly acidified urine was used as a base and a definite amount of purified crystalline hippuric acid (m.p. 185° to 187° C.) was weighed out into a series of beakers. Tenth normal sulfuric acid was added drop by drop until no more precipitate formed, then an additional 1 c.c. of acid was added. The results are tabulated in Table I. This table shows that the amount of 10 N sulfuric acid required to precipitate hippuric acid from 100 c.c. of urine quantitatively is inversely proportional to the amount of hippuric acid present. Thus, the less hippuric acid present, as in beaker 1, the more 10 N sulfuric acid necessary to precipitate it quantitatively. About 76 per cent of the hippuric acid in beaker 5 came down with no addition of inorganic acid; to get the remaining 24 per cent, 2.5 c.c. of 10 N sulfuric acid had to be added.

The estimation of hippuric acid was made by the titrimetric method rather than the gravimetric method recommended by Quick. The gravimetric procedure is out of the question when the precipitation is carried out in saturated salt solution. To rule out the objection raised by White and associates[4] that the precipitate in addition to hippuric acid might contain unconjugated benzoic acid and glucuronic acids and thus give erroneous acidity values by the titration method, the following experiment was made: The titrated hippuric acid solution was filtered quantitatively into a volumetric flask. The beaker and filter paper pulp were washed with distilled water into the flask until no test for chloride ion was obtained, and made up to volume. An aliquot portion was analyzed for total nitrogen and compared with the calculated nitrogen from the hippuric acid titration value. The results are shown in Table II.

TABLE II

SPECIMEN NO.	HIPPURIC ACID BY TITRATION (GM.)	CALCULATED NITROGEN (MG.)	MG. OF NITROGEN BY MICRO-KJELDAHL	% ERROR
1	3.75	293	300	+2.3
2	3.14	246	257	+4.4
3	2.85	223	237	+6.2
4	3.57	280	285	+1.7
5	4.12	322	343	+6.5
6	0.52	40.6	44	+8.3

These data warrant the conclusion that no other titratable acid is precipitated along with hippuric acid, and that the titration method is reliable.

The analytical procedure now used at the Cook County Hospital is as follows: Render the urine* specimen acid to litmus with acetic acid, bring to a boil to remove coagulable material, and filter if necessary. Measure the volume, and if it is more than 150 c.c., concentrate to 150 c.c. and add 30 Gm. of solid sodium chloride per 100 c.c. of urine, i.e., 45 Gm., and heat while stirring until dissolved. Cool to room temperature by immersion in cold water, and while stirring and scratching the sides of the beaker, add drop by drop 1:1 sulfuric acid until no more precipitate forms. (It is difficult to say how much acid to add as different urines require different amounts of acid. In general, it can be said that from 2 to 5 or 6 c.c. are usually sufficient. As a precaution we test each filtrate by adding more acid to see whether precipitation is complete.) Allow to stand for fifteen minutes or more and filter through a fluted filter paper. Wash the precipitate and beaker several times with a 30 per cent solution of sodium chloride (free of sulfate ion), using a fine tip wash bottle until the last washings give negative tests for sulfate ion. Transfer the precipitate with the filter paper back to the beaker in which the precipitation was carried out. Add about 75 or 100 c.c. of distilled water and heat until the precipitate dissolves. Titrate while hot, using 0.5 N sodium hydroxide and phenolphthalein as an indicator.

It was found convenient to report results in terms of sodium benzoate.

Calculations.—

1 c.c. of 0.5 N NaOH = 1 c.c. of 0.5 N $C_7H_5O_2Na$

1 c.c. of 0.5 N $C_7H_5O_2Na$ = 0.072 Gm.

Therefore, X c.c. of 0.5 N NaOH × 0.072 Gm. = grams of $C_7H_5O_2Na$

Solubility correction for 150 c.c. of urine = 0.15 Gm.

SUMMARY

1. The acidity used by previous investigators was insufficient for quantitative precipitation of hippuric acid.

2. The amount of inorganic acid required for a quantitative recovery of hippuric acid varies with the composition of the urine and an excess of acid is desirable.

*If specimen is bile tinted, decolorize with norit by adding 0.3 Gm. per 100 c.c. or more, depending upon the amount of bile in specimen, and boil for about a minute; then filter by suction or fluted filter paper while hot.

3. It was shown that the titration method is reliable as no other organic acids are precipitated with the hippuric acid.

4. A modified quantitative procedure is given in detail.

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THE DETERMINATION OF PHENOL RED WITH THE EVELYN COLORIMETER*

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THE use of phenol red to determine the dilution of test meals by the gastric secretion has been described by Hollander, Penner, and Saltzman.[1] Wilhelmj and Baca[2] modified this method by a correction for very dilute samples: mixing equal quantities of the sample and the original test meal before analysis. The purpose of this report is to record a further modification through the use of the Evelyn colorimeter[3] for making the colorimetric comparisons.

To begin with, the test meal contains 1.0 mg. of phenol red per 100 c.c. instead of 2.0 mg. as used by both Hollander and Wilhelmj. When 5 c.c. of such a concentration are used as a standard, the galvanometer reading of the colorimeter is about 20. On the other hand, solutions containing as little as 0.1 mg. per 100 c.c. give a galvanometer reading of about 85. The Evelyn colorimeter thus gives a much wider working range than is feasible with the Duboseq colorimeter.

Our procedure is as follows: In a 76 by 120 mm. test tube, 5 c.c. of the sample are brought to a definite pink with finely powdered calcium oxide. Then 1 c.c. of 0.5 N sodium hydroxide and 2 c.c. of 0.3 N zinc sulfate are added and the tubes are shaken. After standing for ten minutes, the tubes are centrifuged. Five cubic centimeters of the clear supernatant liquid are placed in a colorimeter test tube and 5 c.c. of 0.5 N trisodium phosphate are added to develop the red color. The colorimeter tubes are then centrifuged for ten minutes at a speed of about 2,000 r.p.m. to throw down the zinc phosphate. The outside of the tube is wiped clean, and the reading is made using a water blank to adjust the galvanometer to 100. The filter used is Rubicon No. 565 because it transmits light of the wave length at which the alkaline form of phenol red has its maximum absorption.[4]

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This method eliminates one step entirely in the Hollander method, since the final reaction takes place in the colorimeter tube in which the reading is made. We have always obtained clear supernatants after centrifuging down the zinc phosphate. The volume of the precipitate is so small that its upper level remains below the light path from the colorimeter lamp.

When we first developed this technique, our studies were confined to water-glucose meals. At this time we prepared blanks by subjecting 5 c.c. samples of distilled water to our complete procedure. These blanks were used to set the galvanometer at 100 before taking our readings. We compared these settings with those obtained by using distilled water without preparation. Repeated tests showed the results to be identical, thus eliminating the need for a blank analysis. With Liebig's beef extract meals, blank analyses were made using the meal without phenol red. Although such solutions have a yellow color, with the filter of wave length 565 mμ, they have a light absorption identical with that of water. We could thus eliminate the blank analyses again.

We have been using this technique for some time and find that the discrepancy between theoretical and observed values is about 2 per cent, or the equivalent of the experimental error of the Duboscq colorimeter. The use of a color filter transmitting light of a wave length which corresponds to the absorption maximum of phenol red in its alkaline form tends to eliminate interference from chromogens which were not removed by the precipitation with zinc sulfate and sodium hydroxide.

We have checked the relationship between the logarithm of the percentage light transmission and the concentration and have found that over the working range of the colorimeter it is linear. It should, therefore, be feasible to eliminate the standard and use a calibration curve. Since the stock solution of phenol red has a tendency to decrease in intensity,[5] we believe the safer procedure is to prepare a standard from the test meal itself and thereby eliminate the possibility of error from such fading or other variation in making up the test meal. In addition to the fact that the use of the photoelectric colorimeter eliminates the obvious subjective factors involved in reading the Duboscq colorimeter, our method has the advantage of considerably shortening the time required to make the determinations.

SUMMARY

A modification of the Hollander method for determining phenol red applicable to the Evelyn colorimeter is described.

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STUDIES ON THE METABOLISM OF IRON AND COPPER*

I. Method for the Determination of Iron and Copper in Blood Serum

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EXPERIMENTS have been conducted in this laboratory for the past seven years to obtain data on the metabolism of iron and copper in the human body. We established the normal values for whole blood iron and whole blood copper for persons of various ages and of both sexes.[1-4] We also reported whole blood iron and copper values in the various anemias and other pathologic conditions.[2, 5]

We found that a reciprocal relationship existed between the iron and copper content of the whole blood in anemias and allied pathologic conditions.[2, 5] When the whole blood iron was decreased, the whole blood copper increased; and likewise when the whole blood iron increased, the whole blood copper decreased. Hypercupremia was the usual response to hypoferronemia.

Recent investigations suggest that the blood serum serves as the medium for transportation of iron in the body.[6-8] Since copper is so closely associated with iron in the hematopoietic process, it is logical to believe that it also may be transported in the serum. Our present study of the iron and copper content of the blood serum was undertaken to gather data relative to the absorption and utilization of these two important biological elements.

The serum normally contains very small quantities of copper and nonhemoglobinous iron. Our first effort then was to establish a satisfactory method for the determination of serum iron and copper. Repeated efforts to determine the iron and copper content in the same sample of serum failed to give reliable results, hence a method was devised for the analysis of aliquot portions of serum separately for iron and copper. The chief difficulty in the latter method is that serum iron in the presence of the copper reagent, sodium diethyl-dithiocarbamate, produces a brownish color which interferes with the yellow color used for the detection of copper. Other workers also have observed this difficulty.[9] Our attempts to overcome this interference by binding the iron with sodium pyrophosphate were successful. The binding effect of sodium pyrophosphate is demonstrated in Table I, where solutions containing known amounts of iron and copper were treated with saturated sodium pyrophosphate, ammonium hydroxide, and sodium diethyl-dithiocarbamate, respectively, and the color extracted with isoamyl alcohol. The colored alcohol layers were transferred to microcolorimeter cups and compared in the Klett colorimeter, using solution I as the standard.

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Obviously since solutions I and VIII (Table I) checked with one another, no contamination was introduced through the use of sodium pyrophosphate. Furthermore, in solutions II, III, and IV (Table I), the experimental values agreed with the actual values, while in solutions V, VI, and VII (Table I) they did not. This fact led us to believe that the binding of serum iron is essential in the sodium diethyl-dithiocarbamate method for the determination of serum copper.

TABLE I

EFFICACY OF SODIUM PYROPHOSPHATE TO BIND SERUM IRON

SOLUTION NO.	FE AND CU PRESENT (MG.)	$Na_4P_2O_7.10H_2O$* (C.C.)	NH_4OH (C.C.)	CARBAMATE (C.C.)	ALCOHOL (C.C.)	COLORIMETER READING (MM.)	COPPER		% ERROR
							EXP. (MG.)	ACTUAL (MG.)	
I	0.010 Cu 0.000 Fe	1	2	2	4	15.00	--	0.010	--
II	0.010 Cu 0.020 Fe	1	2	2	4	15.00	0.010	0.010	0
III	0.010 Cu 0.010 Fe	1	2	2	4	15.00	0.010	0.010	0
IV	0.005 Cu 0.010 Fe	1	2	2	4	30.00	0.005	0.005	0
V	0.010 Cu 0.020 Fe	0	2	2	4	17.60	0.008	0.010	20
VI	0.010 Cu 0.010 Fe	0	2	2	4	15.80	0.009	0.010	10
VII	0.005 Cu 0.010 Fe	0	2	2	4	33.60	0.004	0.005	10
VIII	0.010 Cu 0.000 Fe	0	2	2	4	15.00	0.010	0.010	0

*Redistilled water, free from copper and iron, was used to prepare the sodium pyrophosphate solution.

From the results in this table we concluded that 1 c.c. of a saturated sodium pyrophosphate solution should bind completely the interfering iron present in the samples of serum used for our copper analyses, since our normal serum iron values rarely exceeded 0.020 mg. of iron per 5 c.c. of serum.

PROCEDURE

Reagents and Apparatus.—All reagents and apparatus used in these experiments were rendered free of iron and copper. Acids and water were redistilled from all-glass distillation apparatus until free from iron and copper.* The ammonium hydroxide (28 per cent) was found to be copper free, and consequently it was unnecessary to purify it.† Blanks were run frequently to check the absolute purity of the reagents used. The syringes and platinum needles used for drawing the blood samples were carefully rinsed with redistilled water before sterilization by autoclaving.

Using a sterile syringe, 37 c.c. of blood were drawn between 8:00 A.M. and 9:00 A.M. from donors who had fasted for at least twelve hours. Two 14 c.c. samples were transferred immediately to clean, dry centrifuge tubes and centrifugated. In the transfer of the blood from the syringe to the tubes, the blood was allowed to run slowly down the side of the tube without any external

*Qualitative test for iron: 5 to 10 c.c. of solution to be tested are treated with 1 c.c. of HCl, 0.1 c.c. of concentrated HNO_3, 4 c.c. of 3 N KCNS, and 2 c.c. of isoamyl alcohol. If the alcohol layer is colorless, the test solution is free from iron.

Qualitative test for copper: 5 to 10 c.c. of solution to be tested are treated with 0.5 c.c. of 28 per cent NH_4OH, 2 c.c. of sodium diethyl-dithiocarbamate, and 2 c.c. of isoamyl alcohol. If the alcohol layer is colorless, the test solution is free from copper.

†The ammonium hydroxide was obtained from the Mallinckrodt Chemical Co.

pressure on the plunger. Likewise when the blood was withdrawn from the veins, no suction was exerted on the plunger. This precaution must be taken in order to avoid hemolysis. The remaining blood was transferred to a large test tube containing 15 mg. of sodium oxalate and was used subsequently for the determination of hemoglobin and of whole blood iron and copper, cell volume, and for the making of red and white blood cell counts. The samples from which the serum was obtained were centrifugated at 1,500 r.p.m. for one and one-half to two hours. After centrifugation the serum was carefully removed from the cells and tested for hemolysis.

Testing the Serum for Hemolysis.—Using a 5 c.c. Ostwald-Folin pipette, a sample of serum slightly in excess of 5 c.c. was withdrawn, and the serum allowed to flow into a dry receptacle until the 5 c.c. mark was reached. This serum was employed for the benzidine test. The remaining 5 c.c. were transferred quantitatively to a vitreosil dish.

Benzidine Test for Hemolysis.—To a clean 15 c.c. test tube exactly two drops (equivalent to 0.2 c.c.) of the blood serum for the benzidine test, 1 c.c. of 1 per cent benzidine dihydrochloride* solution, and 1 c.c. of 3 per cent hydrogen peroxide were added. The 3 per cent hydrogen peroxide was prepared from superoxol (30 per cent H_2O_2) by dilution. If a blue color appeared, the benzidine test was positive, indicating the presence of hemoglobin. A serum sample giving a positive benzidine test was not used for serum iron determinations. It was used for serum copper determinations, however, if only a slight blue color was obtained in the benzidine test. Our results have indicated that traces of hemoglobin do not affect the accuracy of serum copper analyses.

We have found this benzidine test capable of detecting hemoglobin in blood which has been diluted 1:100,000; that is, 1 c.c. of this diluted sample containing 1.14 μg of hemoglobin, or 0.0038 μg of hemoglobin iron, gave a positive benzidine reaction. The hemoglobin content of the blood sample was calculated from the iron content of 5 c.c. of whole blood, as determined by the dry ash method. This test was performed on each freshly prepared solution of benzidine dihydrochloride to insure the constant sensitivity of our benzidine test.

Because this test is capable of detecting 0.0038 μg of hemoglobin iron, a negative benzidine reaction in 0.2 c.c. of serum would indicate that there is no more than 1.9 μg of hemoglobin iron in 100 c.c. of serum. This amount of iron falls well within the limits of experimental error, and, therefore, may be considered negligible.

SERUM IRON DETERMINATION

Five cubic centimeters of benzidine negative serum were transferred to a vitreosil dish and evaporated to dryness on the hot plate, with constant stirring to prevent spattering. The contents were covered and ashed for eight hours in an electric furnace. One cubic centimeter of concentrated nitric acid was then added, and the contents were evaporated to dryness on the hot plate. If ashing was incomplete, it was finished at this stage by heating gently over an open flame until all traces of carbon disappeared. Two and one-half cubic centimeters of 6 N hydrochloric acid were added, and the solution was warmed slightly to dissolve the ash completely. The contents were transferred quantitatively to a

*The crystalline benzidine dihydrochloride was obtained from Eastman Kodak Co. A 1 per cent solution of the salt was stable for at least a month.

50 c.c. glass-stoppered cylinder, the dish was washed carefully with redistilled water containing 0.5 c.c. of 6 N hydrochloric acid, and the wash was transferred to the cylinder.

The standard was prepared as follows: Using a 1 c.c. Ostwald-Folin pipette and a standard iron solution containing 10 μg of iron per cubic centimeter, 1 c.c. of the iron solution (equivalent to 10 μg of iron) was transferred to a 50 c.c. glass-stoppered cylinder, and 3 c.c. of 6 N hydrochloric acid were added.

To both standard and unknown 0.1 c.c. of concentrated nitric acid was added, and the containers were rotated gently to insure thorough mixing. Exactly 4 c.c. of 3 N potassium thiocyanate and 4 c.c. of isoamyl alcohol were added. The cylinders were stoppered and shaken for one to two minutes and then allowed to stand until the alcohol layer separated from the aqueous solution.

Using a small pipette, the colored alcohol layer was transferred to a dry acid-washed filter paper, 1 inch in diameter, and filtered into a microcolorimeter cup. Comparison in the colorimeter was made as soon as possible.

SERUM COPPER DETERMINATION

Five cubic centimeters of serum were transferred to a vitreosil dish and evaporated to dryness, with constant stirring to prevent spattering. The contents were covered and ashed for eight hours in an electric furnace. One cubic centimeter of concentrated nitric acid was added, and the contents were evaporated to dryness on the hot plate. To insure complete ashing the residue was heated gently over an open flame until all traces of carbon disappeared. The ash was then dissolved in 2.5 c.c. of 6 N hydrochloric acid, warming slightly to dissolve it completely, and transferred quantitatively to a 50 c.c. glass-stoppered cylinder. The dish was finally washed with redistilled water containing 0.5 c.c. of 6 N hydrochloric acid, and the washings were transferred directly to the cylinder.

A copper standard was prepared as follows: Using a 1 c.c. Ostwald-Folin pipette and a standard copper solution containing 10 μg of copper per cubic centimeter, 1 c.c. of the copper solution was transferred to a 50 c.c. glass-stoppered cylinder, and 3.0 c.c. of 6 N hydrochloric acid were added.

Using redistilled water, both standard and unknown were made up to a volume of approximately 35 c.c. To both standard and unknown 2 c.c. of concentrated ammonium hydroxide were added. The solutions were cooled, and 1 c.c. of a saturated solution of pyrophosphate, 2 c.c. of an aqueous solution of 0.5 per cent sodium diethyl-dithiocarbamate reagent, and 4 c.c. of isoamyl alcohol were added. The contents were shaken for one to two minutes and then allowed to stand until the alcohol layer separated from the aqueous solution. Using a small pipette, the colored alcohol layer was transferred to a dry acid washed filter paper as for serum iron, and filtered into a microcolorimeter cup. Comparison was made in the colorimeter.

Some of the serum iron and copper values which we have obtained using the method outlined are listed in Table II. These samples were obtained from normal male college students, 20 to 30 years old.

TABLE II

WHOLE BLOOD AND SERUM IRON AND COPPER CONTENT OF MALE ADULTS

NAME	RED BLOOD CELLS	WHITE BLOOD CELLS	HEMOGLOBIN* (GM./100 C.C. BLOOD)	SERUM FE MG./100 C.C. SERUM	SERUM CU MG./100 C.C. SERUM	WHOLE BLOOD FE (MG./100 C.C. BLOOD)	WHOLE BLOOD CU (MG./100 C.C. BLOOD)	IRON COLOR INDEX†	CELL VOL. %
Kok	4,384,000	6,500	15.21	0.127	0.114	50.96	0.127	1.18	48.8
Her	4,768,000	13,900	14.35	0.112	0.092	49.08	0.130	1.04	45.8
Cah	4,672,000	5,550	14.35	0.117	0.091	49.08	0.115	1.06	46.0
Gre	4,928,000	7,900	13.19	0.170	0.115	44.20	0.120	0.90	48.0
Cow	4,736,000	5,400	16.02	0.135	0.091	53.68	0.123	1.14	51.2
Rus	5.376,000	5,850	16.23	0.168	0.132	54.37	0.135	1.02	51.2
Mak	4,992,000	5,700	15.16	0.140	0.094	50.80	0.120	1.03	49.1
Sto	4.864,000	12.100	15.91	0.258	0.084	53.32	0.140	1.11	48.0
O'L	5,280,000	7,800	17.19	0.103	0.129	57.56	0.118	1.10	--
He	4,896.000	8,400	14.95	0.170	0.108	50.10	0.121	1.04	46.8
Average			15.25	0.150	0.105	51.30	0.125	1.06	

*The hemoglobin values were calculated from the whole blood iron values, using the Butterfeld factor:

$$\text{Hemoglobin, Gm. in 100 c.c. of blood} = \frac{\text{Milligrams of iron in 100 c.c. of blood}}{3.35}.$$

†Iron color index $= \frac{\text{Milligrams of iron in 100 c.c. of blood}}{\text{First two figures of red blood cell count}}$.

COMMENT

On the whole, the serum iron values which we have obtained using the method outlined (0.103 to 0.258 mg. per 100 c.c. of serum) fall within the range reported by other investigators who used different methods. Moore and his collaborators, from an analysis of fifteen male subjects, reported a range of 0.094 to 0.174 mg. per 100 c.c.[6] Walker[10] reported a range of 0.040 to 0.230 mg. per 100 c.c. from a series of twelve medical students. Fowweather[11] obtained a range of 0.095 to 0.180 mg. per 100 c.c. Other workers, not specifying the sex studied, reported such values as the following for 100 c.c. of serum: Locke, Main, and Rosbash, 0.083 to 0.115 mg.; Warburg and Krebs,[12] 0.067 to 0.116 mg.; Barkan,[13] 0.056 to 0.140 mg.; and Langer,[14] 0.050 to 0.180 mg.

We have observed in an occasional case a marked deviation from the general range of values reported. This suggests the possibility that the absorptive state of the individual and the iron content of his diet for a period of time preceding the analysis are possible factors affecting serum iron concentrations.

The serum copper values we have obtained (0.082 to 0.132 mg. per 100 c.c. of serum) are slightly higher than those of Locke, Main, and Rosbash,[9] who reported a range of 0.073 to 0.087 mg. per 100 c.c. for eight cases, and of Warburg and Krebs,[12] who stated 0.082 mg. per 100 c.c. as their average value for ten adults.

We are continuing our determinations so as to establish a larger series of normals that may give us a more accurate range of normal values which we intend to publish at a later date.

SUMMARY

1. A relatively simple dry ash method has been described for the determination of iron and copper in blood serum. Aliquot samples of serum were evaporated to dryness and ashed in an electric furnace for eight hours; the ash was

treated with nitric acid, dissolved in dilute hydrochloric acid, and transferred to glass-stoppered cylinders. The solution in one cylinder was then analyzed for serum iron, the other for serum copper. For iron analysis the solution was treated with nitric acid, 3 N potassium thiocyanate solution, the color was extracted with isoamyl alcohol, and the unknown was compared with a standard in a colorimeter. For copper analysis the solution was treated with ammonium hydroxide, sodium pyrophosphate, and sodium diethyl-dithiocarbamate. The color was extracted with isoamyl alcohol and the unknown was compared with a standard in the colorimeter.

2. Using this method, values for the iron and copper content of the serum of ten male adults are reported. The serum iron values range from 0.103 to 0.258 mg., giving an average of 0.150 mg. per 100 c.c. of serum. The serum copper values range from 0.084 to 0.132 mg., yielding an average of 0.105 mg. per 100 c.c. of serum.

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MEDICAL ILLUSTRATION

AN INTRODUCTION TO ILLUSTRATION

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WITH ILLUSTRATIONS BY ELIZABETH S. CONE AND CARL DAME CLARKE

IN ESTABLISHING the Department of Medical Illustration in this JOURNAL, I feel that an introduction to illustration is in order. This subject has been discussed somewhat at length in previous articles and books.[1-4] At best this introduction is only a short summary to call to the reader's attention the different types of illustration and how they may be used.

For the purpose of portrayal the scientist has at his disposal three mediums of illustration. The first of these is photography, the second is drawing or painting, and the third is modeling. In this latter medium, molds and casts of the actual subject are employed more than sculptured models. For the purposes of medicine photography is used more than drawing, and drawing is used more than modeling. It is common for the scientist to overlook the possibilities of one medium of illustration in his enthusiasm for another.

There are some cases in which the photograph will serve as a better illustration than the drawing, and other cases in which the drawing is indispensable. The wax or rubber model serves best in the small classroom where it can be used during a didactic lecture. *The medium of illustration to be chosen depends entirely on the subject to be illustrated and the purpose for which the illustration is to be used.* The photograph is the cheaper method and takes less time to produce. Naturally, it is the most popular method of illustration. There are cases in which it is absolutely impossible for the photograph to show to good advantage in comparison with the drawing. There are also instances in which the making of a drawing would be an unnecessary expense and a waste of time when the photograph would be sufficient.

Examples of cases where the ordinary photograph is suitable are as follows: skin lesions in which color is of little importance; deformities of any part of the body that manifest themselves on the outer surfaces; pathologic specimens[5] void of color, as after fixation in formalin; apparatus of various types, such as chemical, surgical, and medical. In many cases pen-and-ink diagrammatic line drawings of apparatus show to better advantage the operation of such equipment.

There are cases in which the best photograph is worthless in comparison with the drawing made by the finished artist. It is not only hazardous, but practically impossible, to obtain a photograph through the small incision that is made

to gain access to the abdominal and thoracic viscera. Even the most proficient photographer, using the best equipment, is likely to fail or refuse to try. Without doubt the mechanics of surgery are best illustrated through the medium of draftsmanship. Even the simple pen-and-ink diagram is often superior to the most perfected photograph in presenting a surgical procedure for mental absorption.

In many cases drawings are superior not only because of the inaccessibility of the field, but also because important structures are often covered by blood, mucus, fascia, fat, and other unimportant parts that prevent a clear view to the lens of the camera. The artist can eliminate these obstructions from his drawing, but it is generally impossible for the photographer to do so in his photograph. Of course, photographs can be retouched, but when any great amount of retouching is done the photograph becomes more of an art job than a photograph. To this end it would have been better for the artist to have made the entire illustration.

In comparing the drawing and the photograph made through the microscope, it often becomes a matter of personal taste on the part of the physician as to his preference. I am of the opinion that the average physician is more accustomed to poor photomicrographs than he is to good ones. Not all physicians are fortunate enough to have at their disposal trained medical artists and photographers. In fact, the making of medical pictures is often left to artists and photographers doing the usual run of commercial work. Few, if any, commercial photographers have the equipment for making photomicrographs, and since this is a highly developed and specialized field of photography, few of them understand how to obtain the best results. Therefore, photography of this type is left to the pathologist or laboratory worker, who may be very proficient in his own field but a poor photographer. He may consider his results good until a comparison with truly excellent photographs is made.[6, 7]

The photomicrograph of a few years ago was a poor excuse for a good drawing made at the same time of the same subject. With the continued development and use of panchromatic photographic plates, color filters, apochromatic lenses, and perfected photomicrographic apparatus, the drawing of the microscopic subject is beginning to recede into the background. Photomicrographs of any microscopic section can be made through any known and used power of lenses. In other words, low-power pictures covering the entire section can be produced as well as the pictures made through the oil-immersion lens. Any degree of magnification between these two can be successfully obtained and the section photographed at that specified power. Magnifications between those given by combinations of specified oculars and objectives may be obtained by extending the bellows of the photomicrographic camera.

It is possible for the artist to accentuate any part of the cell structure as well as "hold back" the insignificant parts in a microscopic slide. The photographer can do the same thing by the use of the proper color filter or filters, with the correct plate and slight variations in exposure, and development of the negative and positive. Small sections of a negative may be accentu-

ated by the use of a dye on other parts of the negative to hold them back during the printing, or by the use of tissue paper beneath the negative in the printer. It stands to reason that the photograph is a truer representation of the original. However, in high-power work detail and depth in a cell are often difficult to obtain by photography. The artist can improve on some photomicrographs because he can make a composite picture of what comes in and out of view as he focuses his microscope up and down. A high-power photomicrograph may be good as far as the photographic technique is concerned, but a drawing of the same subject can be superior because of the ability of the eye and hand to record on paper in a single picture the various depths of a microscopic section.

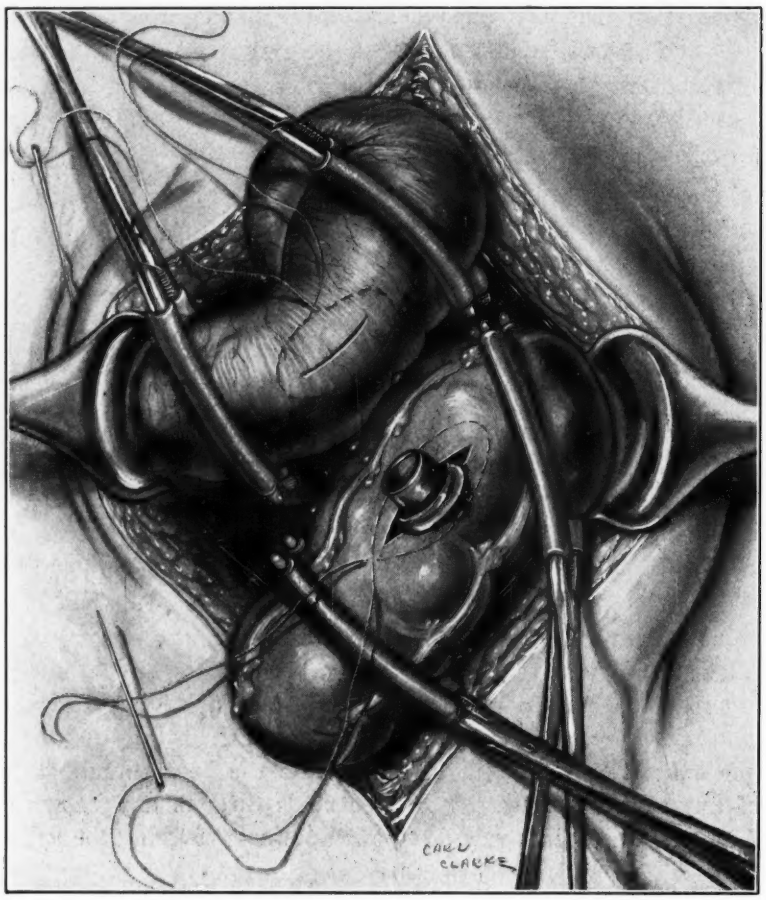

Fig. 1.—A typical half-tone drawing for the illustration of the mechanics of surgery.

It is possible to combine drawing with photography to produce a drawing or a picture composed of both drawing and photography. The silver print or photochemical method of combining drawing and photography is a procedure that can be applied to the making of any type of drawing. However, it is more suitable for pen-and-ink illustrations. It can be used for the wash, water color, oil color, tempera, and even pencil dust techniques. This subject has already been discussed fully[8] and will not be dealt with in this introduction.

Natural color photographs can be made and used for lantern slides or publication. These pictures in color are made on glass or celluloid film and are often exceptionally well done. As a rule, the natural color photograph compares favorably with the best color drawings used in scientific publications. However, the artist has at his disposal many colors from which he may make hundreds of combinations. The natural color photograph is generally composed of three

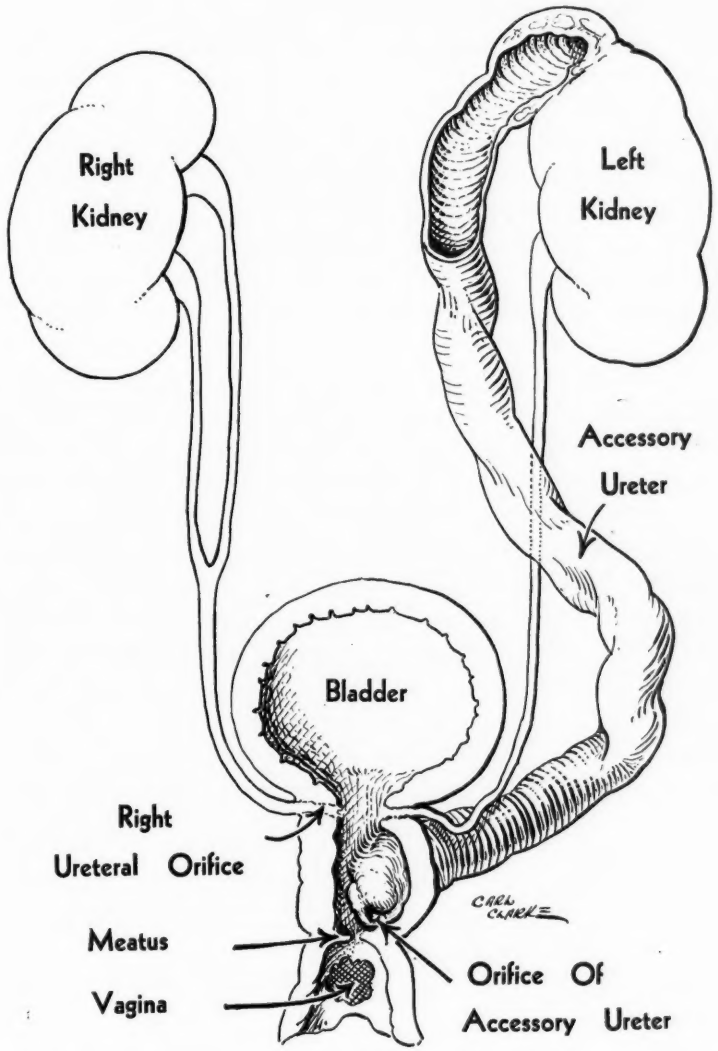

Fig. 2.—Sometimes a simple pen-and-ink semidiagrammatic sketch serves best as a drawn illustration.

colors; therefore all colors must be those that are originally in the emulsion or the combinations that can be obtained by optically mixing these colors. A good color photographer will invariably produce a better color picture than a poor artist and, in turn, a good artist will produce a better color picture than a poor photographer.

The artist may be able to color or tint photographs, but the drawing or painting by the skilled artist without photographic aid is usually superior. Photographs to be colored or tinted as medical illustrations should be made first in sepia tone. This is necessary as the black undertone has a tendency to "kill" or destroy the brilliancy and strength of warm colors such as reds and yellows. These colors are used in painting or glazing over the photograph.[9]

One of the important factors to be considered before having full-tone colored pictures made, whether colored photographs or paintings, is the expense of reproduction. This expense is augmented by the necessity of making numerous photoengraved cuts, one of each color, and the added processes of printing. Many medical journals require the author, or the institution with which he may be affiliated, to bear the cost of color reproductions. However, the cost of full-tone color pictures is greatly reduced by using flat color only. Structures such as veins, nerves, and arteries, can be accentuated by the use of flat color over black-and-white half tones or line drawings. The color illustrations in the *Hand Atlas of Human Anatomy* by Spalteholz are examples of the use of flat color in photoengraving. The average magazine cover is generally reproduced in full tone.

It is my assumption that better reproductions in black and white are obtained from photographs or drawings that are themselves in black and white rather than in color. For this reason, if the picture is not to be reproduced in color, the artist or photographer should make the original in black and white.[10]

Practically all lantern slides are made by the photographic process, regardless of what the original may be. One exception to this rule is when the diagram or sketch is drawn directly on a prepared glass of lantern slide size; another is the process of typewriting through carbon paper placed next to a piece of cellophane or celluloid. This latter method gives a carbon impression on the cellophane, which is then mounted between two lantern slide cover glasses for projection.[11]

The same problems, as well as many others that arise in still photography, apply to motion picture photography. The scientist should write out a scenario before proceeding. This is of considerable importance in making excellent professional motion pictures. Generally a picture is taken of a surgical operation or similar procedure. After the film is developed, titles are inserted or the film is scored with sound. Few scientific films tell their stories as clearly or coherently as a logical sequence of events.[12]

The scientist should bear in mind that the preparation of a motion picture is comparable to the preparation of an article for publication. An outline or scenario is made, the material is gathered and then photographed, the negative is edited, and finally the negative is titled or scored with sound and printed. This produces a continuous film without patches which cause noise and shaking on the screen. Most persons prefer to edit a positive print, then make the negative correspond to the positive. However, if the editing is done on the negative, the expense of an additional positive is eliminated. A scientific subject that is worth recording is usually worth making into both the negative and the positive rather than reversing the negative into the positive, which is the

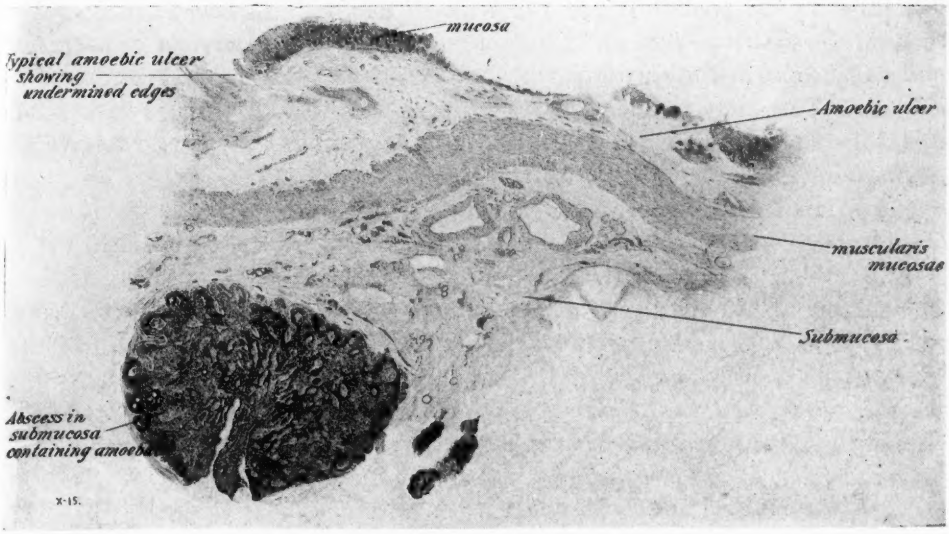

Fig. 3.—In the past most microscopic subjects were drawn, as is shown in this illustration.

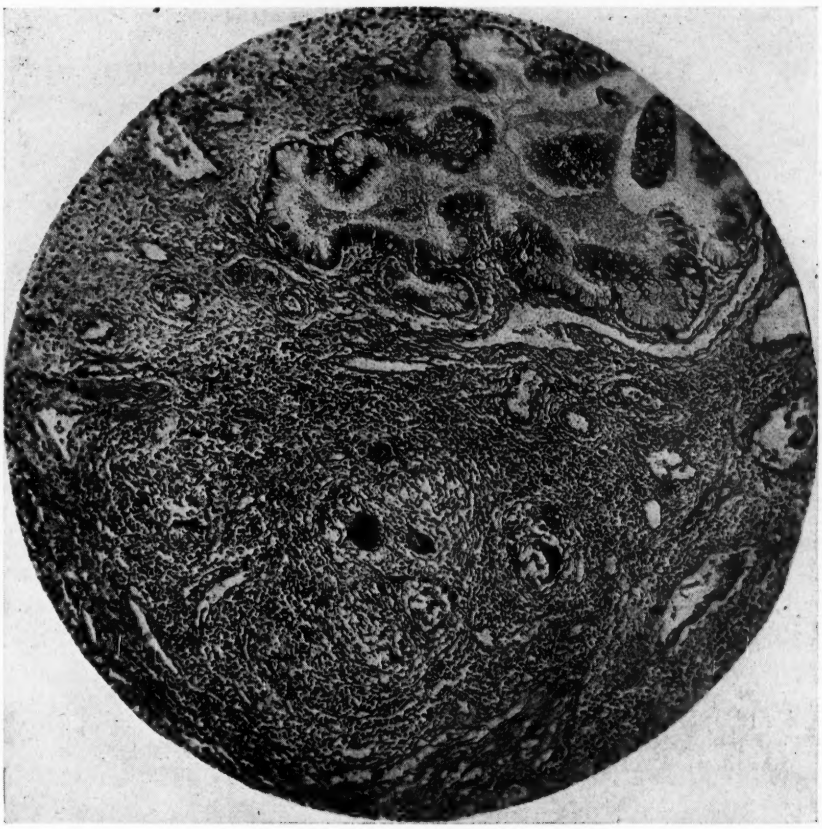

Fig. 4.—The above photomicrograph of a case of tuberculosis of the cervix may be used for comparing with the drawn microscopic picture in Fig. 3.

general rule. It is slightly more difficult to follow the action on the negative than on the positive. The difference in price between Kodachrome or color film and panchromatic film warrants the use of color film entirely for most scientific subjects. This positive color film should be edited, titled, or scored with sound, and immediately duplicated before it has been run long enough to receive annoying scratches.

Animated drawings are playing an important part in scientific illustration. Their production is so highly technical that it becomes a specialized field within itself. The artist who intends to make acceptable animated drawings should, in addition to his knowledge of art, understand the fundamentals of photography, especially motion picture photography. These fundamentals embrace lens action, such as focusing, focal length, and speed of lenses, as well as exposure; the mechanics of the time lapse motion picture camera; and, finally, the technique of the processing room, for it is here that many interesting and necessary animated effects are produced with chemicals and the printing machine.

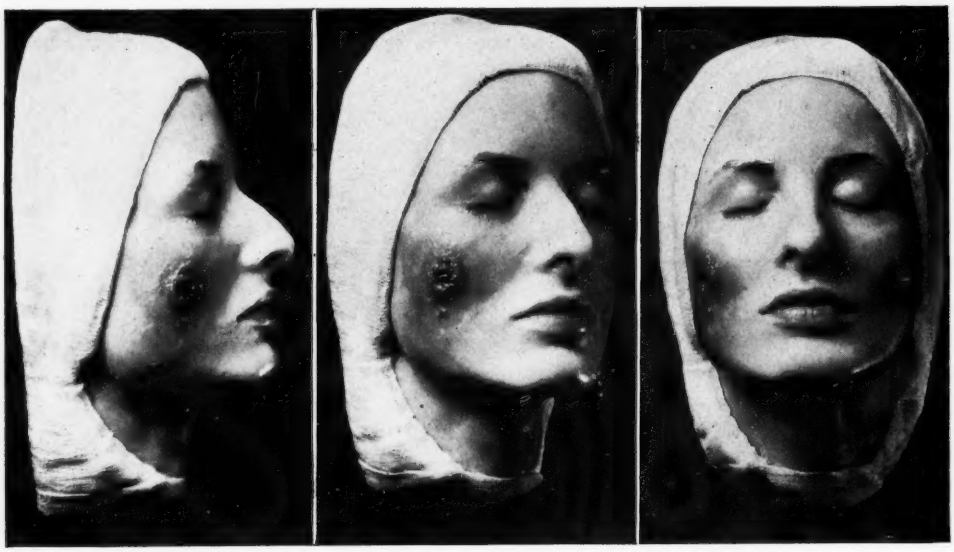

Fig. 5.—The three-dimensional wax cast serves best for museum purposes and for study in the small classroom.

These, of course, apply only to the artist who does his own camera work. However, it is advantageous to learn the fundamentals of the entire process. Photography and drawing are so interdependent for the animation worker that a description of one is inadequate without a description of the other. The motion picture, when properly conceived, photographed, and edited, becomes man's most powerful tool for the dissemination of knowledge. Animation provides a means of obtaining motion pictures which cannot be produced by ordinary cinematography. In other words, animated drawings accomplish the same purpose for the motion picture that an ordinary drawing accomplishes for the textbook. It seems evident that animated drawings will play an even greater part in the dissemination of scientific knowledge than they do at present. This is indeed a fascinating branch of scientific illustration and will not only be in-

teresting to the artist and photographer, but will also tax his ingenuity and imagination in developing new processes and equipment.[13]

Wax or plaster casts can be made of skin lesions, deformities, and abnormal growths and depressions of the body. These molds and casts are made by first applying liquid plaster of Paris or an agar composition to the properly prepared surface. When these materials have set and are removed, they constitute the mold, which in turn is filled with plaster, wax, celluloid, or rubber. The mold is removed after the hardening of the positive material, thereby producing an exact impression which shows such details as the pores of the skin, provided the work has been done properly.

The agar composition is pliable enough to permit free removal of the mold from the anatomic part in cases where there is a degree of undercutting. The agar composition[14] is more suitable for use on the living body or on fresh structures after removal from the body. For the inanimate object composed of plaster or wax the rubber mold serves best. The simplest rubber molds are made from prevulcanized latex combined with cellucotton.

By the use of molds and casts lifelike reproductions can be made of extremities, skin conditions, or any part of the abdominal or thoracic viscera after its removal from the body. These casts can be colored to represent the originals and often have been mistaken for the actual organ or part.

Cut or destroyed parts of specimens can be reconstructed before casting. If the worker is clever, he can model or sculpt the entire subject in clap, wax, or plasteline. From his original work a rubber or plaster *piece mold* can be made to reproduce a number of casts in colored wax or plaster.[4]

Casts are considered to be the best representation of the original but are more cumbersome to handle in exhibiting than the photograph or drawing. Photographs and lantern slides can be made from them for reproduction or projection. Wax or plaster casts serve best as museum pieces. They can also be used for student instruction in small groups.

The possibilities of moulage making are unlimited. Its value to the human race warrants further study and rapid propagation. As an example, consideration should be given to the crude, artificial anatomic appliances which are attached to the body in the vain hope that they will make the unfortunate wearer happier, more presentable to society, and better equipped to cope with the ever-mounting competition of commercial, social, and economic existence. It is indeed deplorable that such prostheses have not been perfected to a degree that would produce realistic, natural, and comfortable appliances of practical use. The present-day artificial limbs are examples of prostheses that lack realism. False teeth have been perfected to a greater extent. There is little reason why other artificial parts should not be developed to the same degree.[4, 14, 15]

An effort should be made to establish a technique in its most simple form. When this is accomplished, other incomplete fields may be conquered and additional knowledge may be gained. . In fact, new ideas will develop and encourage the worker to return to the original problem in order to raise it one step nearer the unattainable peak of perfection. The goal is unlimited but the zest of trying is worth the effort.

In conclusion, it may be repeated that the subject to be depicted and the purpose for which the illustration or reproduction is to be used determine the medium through which it should be produced. It will be well worth the physician's time to discuss this matter fully with the artist and the photographer before he blindly orders a paper to be illustrated. It is hoped that such a discussion will improve medical illustrations and their worth in conveying ideas from one person to another.

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Erratum

On page 554 of the December number of the JOURNAL in the article by Vera B. Dolgopol, M.D., and Helen Y. Markus, M.T., entitled "Rapid Diagnostic Method for Testing the Virulence of Corynebacteria," the second sentence under *Virulence Test* should read:

"A similar amount was injected *intracutaneously* into another guinea pig which was used for the same number of control tests, and 500 units of diphtheria antitoxin was given *intraperitoneally*.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SYPHILIS, Latent, A Serologic Verification Test in the Diagnosis of, Kahn, R. L. Arch. Dermat. & Syph. 41: 817, 1940.

A serologic test is presented in this article which should help to detect false positive reactions obtained in the serodiagnosis of syphilis. The test is based on experimental studies which indicate that sera of nonsyphilitic persons which give positive reactions possess characteristics that can be differentiated from those possessed by sera of syphilitic persons. The technique of the test is similar to that of the standard Kahn test, except that it is carried out at 37° C. and at 1° C. The technique is applicable in questionable cases of syphilis in which sera give doubtful or positive reactions with a diagnostic test.

The method follows:

In the performance of the verification test, a thorough knowledge of the Kahn test is necessary. In a broad sense, the verification technique consists in performing Kahn tests at 37°, 21°, and 1° C. Since such tests are routinely carried out at approximately 21° C., the basic differential temperatures are 37° and 1° C.

In carrying out the technique at 37° C., the reagents as well as the glassware must be properly warmed, and the reagents must be mixed under warm conditions. 1. Kahn racks and tubes are warmed by placing them in a 37° C. water bath fifteen minutes before performing the test. The pipettes are kept in a 37° C. incubator. 2. Standard Kahn antigen is mixed at room temperature in the usual manner with saline solution according to the titer. The antigen suspension is then transferred to a Kahn tube, stoppered and placed in the water bath for fifteen minutes. 3. The serum is heated for thirty minutes at 56° C. (132.8° F.) and is then placed in the 37° C. water bath for fifteen minutes before being pipetted into the antigen suspension. 4. The amounts of antigen suspension (0.05, 0.025, and 0.0125 c.c.) are deposited at the bottom of the Kahn tubes, followed by the serum (in 0.15 c.c. quantities), and the mixture is shaken for ten seconds without removing the rack from the water bath. The racks remain in the bath from five to ten minutes and are then shaken for three minutes under standard conditions. 5. Physiologic solution of sodium chloride, kept in a 37° C. water bath, is then added to each tube in the usual amounts (1, 0.5, and 0.5 c.c.); the tubes are agitated sufficiently to assure proper mixing, and the results are read immediately. 6. A microscope mirror or a slit lamp is used in the reading of results. The precipitation readings in each tube are recorded in the usual manner.

The technique at 1° C. requires an ice-water bath, which can be easily improvised with chopped ice. A square, flat-bottomed pan, 6 inches (15 cm.) or more deep and large enough to hold several racks, will be found adequate. Every step in the technique as carried out in the 37° C. water bath is applicable to that in the 1° C. water bath. It is important not only to bring the temperature of the serum and antigen suspension to 1° C. but also to see to it that the serum is added to the antigen suspension and mixed at this temperature. Tests performed at 1° C. may require special care in reading owing to the film of moisture that condenses on the outside of the tubes. This film may be wiped off with a cloth and the results read as previously indicated.

In the experiments described in this article the three-minute shaking of the tubes was carried out at room temperature. In experiments to be undertaken it is planned to determine the effects of shaking at 37° C. and at 1° C.

It is suggested that no more than ten tests be carried out at any one time, either at warm or at cold temperatures.

In view of the fact that the basic value of the test under consideration lies in the difference in the precipitation results at 37° and 1° C., it is evident that technical steps which do not adhere strictly to these two temperatures will neutralize the value of the results.

GLUCOSE, Renal Threshold for; Normal and in Diabetes, Lawrence, R. D. Brit. M. J. May 11, 1940.

The study of the renal threshold in diabetic and normal persons with healthy renal function shows frequent upward and downward deviations from the accepted "normal" figure of some 170 mg. per 100 c.c.

The conception of a "normal" threshold is false. There is an *average* renal threshold, just as there is an average blood pressure, and the many deviations from it should be looked upon as physiologic—a matter of individual idiosyncrasy, and of no pathologic significance.

High and low thresholds are frequent and usually persist at the same level throughout life. A few factors outlined above affect the threshold temporarily or even permanently.

The stigma of something abnormal (and hence pathologic) that has been imputed to a high or low renal threshold should be removed.

BRUCELLA: The Presence of a Capsule on Brucella Cells, Huddleson, I. F. J. Am. Vet. M. A. **96**: 708, 1940.

Capsules have been demonstrated by the method following:

A dilute suspension of Brucella cells is prepared in physiologic salt solution or distilled water. A small drop of the suspension is placed near one end of a clean microscope slide, and drawn across the slide by placing the end edge of another slide at one angle in front of the drop. The film of bacteria is dried in the air. A small drop of Keuffel and Esser India drawing ink No. 3000 is placed back of the beginning of the previous smear and drawn over it with the end edge of another slide. The ink smear is allowed to dry in the air. The smear is now treated with methyl alcohol containing 0.2 per cent glacial acetic acid for five seconds and again air-dried. The slide, film up, is placed on a rack and stained five minutes with aniline crystal violet previously diluted 1:1 with distilled water. The excess stain is now washed off with distilled water and the smear is air-dried.

On examining the smear under the microscope (oil-immersion objective, 10 or 12 × ocular), it is necessary to move the slide to an area where the ink smear is not too dense in order to view the encapsulated bacteria in their normal size. The soma appears as a deep purple coccus or rod inside the capsular material, which stains light pink or purple. If the smear is fixed for five seconds with 5 per cent acetic acid prior to staining, the capsular material does not stain but appears as a light halo around the soma or bacterial body. The size of the capsule depends upon the size of the soma. The size varies from 1.5 to 2.5 microns.

The capsule appears larger in recently isolated cultures and in those that have grown on culture media for not longer than seventy-two hours.

Cultures that have become completely dissociated or rough do not show a capsule.

WEIL'S DISEASE in Hawaii, Tokuyama, S. J. A. M. A. **114**: 2195, 1940.

Twelve cases of hemorrhagic spirochetal jaundice were observed in Hawaii, the diagnosis being confirmed clinically in two cases and bacteriologically in nine cases. The disease was proved to be identical with that occurring in Japan by both clinical and bacteriologic criteria.

Nine of the twelve patients were inoculated with the *Leptospira icterohemorrhagiae* serum of Inada and Ido; seven of the inoculated patients were cured and two died. Death was due to the delay of the serum injection.

Three of the patients did not receive the serum; of these, two died but one recovered.

DARKFIELD, A New Technic for, Sizemore, T. Am. J. M. Technol. 6: 120, 1940.

The ulcer or lesion to be examined is cleansed carefully with sterile normal saline or Ringer's solution, removing all debris and pus. The ulcer is then dried by the application of ether. A sufficient amount of serum for examination may be obtained by exerting general pressure upon the sides of the ulcer or lesion. Place the top of an ordinary blood coagulation pipette to the serum and tilt until enough of the serum is drawn into the pipette. Transfer to a clean slide using a small rubber bulb to expel the serum. Place a cover glass over the serum immediately and compress it slightly. Mount as usual in cedarwood oil.

URINE, The Formation of, Edwards, J. G. Arch. Int. Med. 65: 800, 1940.

In the kidneys of all classes of vertebrates the proximal convolution is primarily secretory and is the only part of the tubule invariably present. The structure and functions of the tubule, when viewed phylogenetically and when studied in health and in disease, would seem to have an importance much greater than is at present demonstrable in detail. Comparative renal studies dealing with the aglomerular kidney, and inaugurated by the author in this connection, have been productive of much interest in secretion by the tubule. It is hoped that eventually the actual role of the tubule in urine formation will be established.

The phylogenic development of the corpuscle as the site of filtration is related to that of the distal convolution as the site of resorption. Filtrable substances in the plasma are excreted by the corpuscle regardless of possible depletion of body fluids and consequent damage to the tissues. The distal convolution, aided in birds and mammals by the thin segment and the ascending limb of the medullary loop, performs by resorption the function of salvage. The hypotonic urine of cold-blooded animals indicates that there has been resorption of relatively more of certain solutes than of water; the hypertonic urine of warm-blooded animals indicates the opposite.

A filtrable fraction of plasma is excreted into the lumen of the tubule by the corpuscle and by the cells of the proximal convolution. Minimal amounts of protein are present in this fraction because the passage of larger amounts is prevented by the epithelial wall adjacent to the glomerular and peritubular capillaries. Many factors so affect filtration by the corpuscle, however, that albuminuria readily develops. The extreme sensitiveness of this structure to such normal or pathologic changes as those which occur in the chemical composition of plasma or in blood pressure flow or volume is responsible for confusing variations and dubious interpretations of its activity.

Diuretics are initially and chiefly effective by their direct or indirect action on one or more of the components of the cardiovascular system. Neither diuretic urine nor the subnormally concentrated urines formed after renal injury should be regarded as necessarily indicating impairment of the resorptive power of the tubule. Urines of low specific gravity may be variously formed as a result of excessive filtration, of decreased resorption of water, or of increased resorption of solutes.

It is not yet established that the formation of urine is primarily the result of filtration and resorption or that resorption is the chief function of the tubule as a whole.

SHOCK: Circulatory Failure of Capillary Origin, Moon, V. H. J. A. M. A. 114: 1312, 1940.

Circulatory failure of capillary origin produces a clinical syndrome which is highly characteristic. This is accompanied by an equally characteristic group of abnormal physiologic and biochemical features. These constitute the clinical syndrome of shock.

The gross and microscopic changes seen post mortem are equally significant. These consist of evidences of capillary damage, such as dilatation, stasis, petechial hemorrhages, edema, and effusions, present in extensive visceral areas. These features are etiologically related to the mechanism by which circulatory deficiency of capillary origin develops.

Hemoconcentration is a highly valuable clinical sign indicating the early stages of this condition. It occurs before other signs of circulatory deficiency are manifest.

The mechanism of this type of circulatory failure includes the reciprocal effects of two major factors—capillary atony and tissue anoxia. Either of these factors will presently cause the development of the other. This reciprocal action gives the circulatory deficiency a self-perpetuating quality which tends toward an irreversible condition.

Few conditions of disease present a more characteristic group of clinical features, physiologic abnormalities, and morphologic visceral changes. This syndrome, if caused by a single etiologic agent, would constitute an entity among diseases. But the origin of this syndrome is highly diversified. It may originate from the numerous and varied agents and conditions which may injure directly the capillary endothelium, from loss of blood and/or fluid sufficient to produce systemic anoxia, from agents or conditions which reduce the volume flow of blood below physiologic limits, from asphyxia of external or internal origin, or from various combinations of the conditions mentioned.

Efforts to combat this type of circulatory deficiency will be directed toward the recognition and removal of the cause and toward interrupting the operation of the vicious circle. If the former cannot be accomplished, the latter will be ineffective.

Until some agent is found which will prevent or relieve capillary atony, efforts to interrupt the cycle should be directed toward the restoration of blood volume and toward relieving the anoxia. The tendency to progress toward irreversibility requires early recognition and action. Hemoconcentration is recommended for recognizing the early stages of circulatory deficiency of this type.

URINE: Control of Urinary Reaction, Bridges, M. A., and Mattice, M. R. Am. J. M. Sc. 200: 84, 1940.

The urinary pH cannot be consistently changed at will without regard for its natural trend.

The acidifying or alkalinizing effect of test substances cannot be judged solely by the urinary pH observed. Comparison with the fundamental or natural curve is necessary.

The natural acid-base curve can be distorted by dietary factors with variable ease in different subjects.

The determination of urinary pH following meals is of no practical clinical value.

It has not been possible by dietary measures to produce continuously alkaline urine.

Temporary elevation of urinary pH is most readily secured with cantaloupe.

The easiest means of maintaining acid urine in the normal individual is the substitution of cranberry juice for water as such in the diet.

The effective action of acidifying drugs is conditioned by the amount of water simultaneously claiming excretion.

Although concentration of urine is regularly associated with increasing acidity and dilution with increasing alkalinity, the normal kidney can excrete a highly acid dilute urine and a definitely alkaline concentrated urine. The elimination of water is not constant throughout the day, thus influencing the urinary pH.

Emotional and mental states demonstrably affect urinary pH.

PNEUMONIA, Sabin Agglutination Test as a Control of the Sulfapyridine Treatment of, Fox, W. W., Rosi, R., and Winters, W. L. Am. J. M. Sc. 200: 78, 1940.

Fifty adult patients with pneumococcic lobar pneumonia treated with sulfapyridine were studied to determine the time at which active immunity first appeared.

All patients with proved type-specific pneumococcic pneumonia developed strongly positive agglutinins at some time in the course of their illness.

Strong type-specific agglutination occurred in the uncomplicated cases on an average of 8.3 days after the onset of the disease; in complicated cases it occurred on an average of 12.5 days.

When active immunity did not appear early in the second week of the disease, complications or delay in resolution frequently occurred.

Persistence of a strongly positive agglutination for the homologous type pneumococcus was a favorable prognostic sign.

Occasionally, empyema developed after a strongly positive agglutination appeared. All such cases, however, had bacteriemia on admission and in some cases this persisted for twenty-four hours or longer after sulfapyridine therapy was begun.

The authors believe that recovery from pneumonia in patients treated with sulfapyridine requires: (1) adequate blood sulfapyridine concentration, (2) maintained leucocytosis, and (3) development of active immunity by the patient in response to his infection.

In uncomplicated pneumococcic pneumonias a safe rule appears to be to continue sulfapyridine therapy at a dosage which will produce a blood level above 4 mg. per 100 c.c. until a strongly positive microscopic agglutination test is obtained with the patient's serum against pneumococci of the type with which he is infected. In complicated pneumococcic pneumonias sulfapyridine should be continued, if possible, until the temperature and pulse have returned to normal, even though strongly positive agglutination has occurred prior to that time.

TRICHINOSIS, Incidence of, in 300 Autopsies at the University Hospital, Ann Arbor, Michigan, Catron, Lloyd. Am. J. Hyg. **1**: Sec. C, 1940.

Forty-four instances of trichinosis (14.7 per cent), all unrecognized clinically, were found by examination of diaphragms obtained from 300 autopsies at the University of Michigan Hospital. All the infestations were shown by digestion of samples of 50 Gm. or less; three were found by examination of a single microscopic section from each diaphragm; and eight were found in the 270 cases in which 1 Gm. of diaphragm was examined microscopically. The digestion method, although demonstrating every infestation, proved inadequate to show the total number present when the larvae were dead and calcified.

TISSUE: Pathologic Changes Observed in Human Tissues Subjected to Subcritical Temperatures, Smith, L. W. Arch. Path. **30**: 424, 1940.

In this paper a brief summary of the pathologic studies in a series of some 60 cases in which patients with cancer were subjected to local or general reductions of temperature and in which autopsies were made is presented. The studies are divided into three major groups: first, those relating to the normal body tissues; second, those relating to tumor tissue subjected locally to temperatures of 40° to 50° F. for varying periods of time; and, third, those relating to metastatic tumor tissue in patients whose general temperature had been reduced to subcritical levels between 74° and 90° F.

Relating to the first group of non-neoplastic or "normal" tissues, it may be said "a priori" that significant changes are the exception rather than the rule. In the series of 60 autopsies marked myocardial degeneration was found in 5 patients. In 3 of these it was associated with definite sclerosis of coronary arteries. In 3 patients acute pancreatitis was seen; these patients had been accustomed to take relatively large amounts of morphine. In 4 patients a significant and persistent fall in blood pressure was observed. In 3 of these there was extensive bilateral adrenal metastasis. Bronchopneumonia other than the terminal event commonly encountered in cases of advanced malignant disease appeared chiefly in the postrefrigeration period, in association with metastatic involvement of the lungs. Its occurrence with and its relationship to refrigeration, accordingly, is particularly difficult to evaluate.

With respect to the second group, temperatures of 40° to 50° F. applied locally to tumors regularly produced regressive changes going on to actual necrosis, even to the point of histologic clearance of the tissue of tumor cells in occasional instances.

In regard to the third group, the changes which were observed in metastatic tumor tissue from patients who were submitted to general reductions of temperature to 74° to 90° F. were similar in kind but varied greatly in degree in comparison with those resulting from application of the lower temperatures locally. In no case were regressive changes encountered until ninety-six to one hundred and twenty hours of refrigeration had been given, and in some cases no significant change seemed to occur even after three hundred hours of such low temperatures.

HYDATIDIFORM MOLE, Genesis of, Hertig, A. T., and Edmonds, H. W. Arch. Path. 30: 260, 1940.

A series of 1,027 spontaneously aborted ova and 74 hydatidiform moles has been studied with respect to the genesis of hydatidiform degeneration. Hydatidiform degeneration to some degree is common, occurring in 40 per cent of these spontaneously aborted ova, or in 4 per cent of all pregnancies. Typical stages in the evolution of a hydatidiform mole are described. A classic hydatidiform mole is uncommon, occurring only once in 2,062 full-term deliveries in this clinic.

Pathologic ova, of ten and two-tenths weeks' mean menstrual age, constituted 47.4 per cent of the total number of spontaneously aborted ova; 66.9 per cent of the pathologic ova showed early hydatidiform degeneration of the chorionic villi. "Nonpathologic" ova, of fifteen and four-tenths weeks' mean menstrual age, constituted 52.6 per cent of the total; 11.6 per cent of such ova showed early hydatidiform degeneration of their villi.

Hydatidiform degeneration of the chorionic villi of pathologic ova begins, in all probability, at about the fifth week of pregnancy, the time when the fetal circulation should begin to function. The fetal circulation in the chorion of the pathologic ovum fails to function because of extreme defectiveness or absence of the embryo. The vascular "anlagen" disappear coincidentally with the onset of hydatidiform degeneration, both processes being a function of absence or of defectiveness of the circulation.

Hydatidiform degeneration is prone to occur in the villi of early pathologic ova because the stroma of the villi is normally loose and their chorionic epithelium is normally active. Hydatidiform degeneration in the villi of early pathologic ova is an expression of continued physiologic activity of the trophoblast (absorption and/or secretion), with resultant accumulation of intravillous fluid, which cannot be utilized because of the lack of a functioning fetal circulation.

Hydatidiform degeneration is less likely to occur in "nonpathologic" ova because of a functioning fetal circulation of some weeks' duration, relatively dense stroma, and relatively inactive chorionic epithelium.

Transitional moles, of sixteen and six-tenths weeks' mean menstrual age, are usually typical pathologic ova containing no embryos, or at most extremely defective ones, although a few contain macerated normal embryos. Typical hydatidiform moles, of seventeen and four-tenths weeks' mean menstrual age, are true pathologic ova possessing no embryos or rarely a very defective one. A typical hydatidiform mole is, therefore, derived from a true pathologic ovum in which the embryo was either absent or very defective from the beginning and which, for reasons unknown, failed to abort at the usual time. Hence, it constitutes a type of "missed abortion."

BLOOD, Significance of Polymorphonuclear Toxic Index in Affections of the Musculo-Skeletal System, Sutro, C. J. Bull. Hosp. for Joint Dis. 1: 61, 1940.

In a survey of approximately 8,000 patients with affections of the musculo-skeletal system, it was found that, in certain cases, the estimation of the toxic index gave additional clinical information, especially in regard to differential diagnosis and prognosis. Since the evaluation of the toxic index is a simple procedure, its application to problems of differential diagnosis and prognosis is warranted.

(The index is equal to number of polymorphonuclear cells showing toxic granules divided by total number of polymorphonuclear cells and the product multiplied by 4.)

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Experimental Poliomyelitis*

THE neutralization test is a method for determining the ability of immune serum to inactivate virus in vitro—in this instance, that of poliomyelitis. In their thorough review of the literature, the authors point out that though much information can be obtained from the test, the discrepancies and irregularities in results interfere with its effective application. The test is of practical value where qualitative determinations are desired, but it is unsuitable for quantitative studies.

In the second portion of the book, which is given over to a description of the author's carefully controlled experiments, a search was made for sufficient data for the development of a more refined and standardized technique. Unfortunately, their goal was not attained.

The final conclusions of the authors are that the test is a specific antigen-antibody reaction, but its presence does not necessarily imply resistance to infection. Since with the test in its present form quantitation is not a practical procedure, improvement or replacement of the neutralization test is desirable for the advancement of immunologic knowledge in poliomyelitis.

Neoplastic Diseases†

THE fact that this classic has now come into its fourth edition attests to the position it holds in the field of medical texts. The author has considerably revised many sections and completely rewritten some chapters.

The opening section on general oncology should be read by all doctors who have occasion to see malignancies, whether they be primarily interested in medicine or in surgery. The chapters on the nature of cancer and on experimental cancer research are particularly interesting.

The second, larger section on special oncology is a most complete and scholarly treatise on tumors of all types.

A book that should be in every physician's personal library, it is of equal value to the medical student, the internist, the surgeon, and the pathologist.

An Introduction to Biochemistry‡

IT IS of distinct benefit to the clinician from time to time to read through a book of this caliber. Although a small volume, it is comparatively comprehensive. The point of view entertained throughout is that biochemistry is an independent discipline related to the physiologic group on the one hand, and pure chemistry on the other.

*Experimental Poliomyelitis. I. A critical review of the literature with special reference to the use of the neutralization test in immunological studies. II. The Neutralization Test. A study of some factors involved in the neutralizing action of immune serum against poliomyelitis virus. By Morris Schaeffer, Ph.D., and Ralph S. Muckenfuss, M.D., Department of Health, Bureau of Laboratories, New York, N. Y. Cloth, 158 pages. Published under the Auspices of The National Foundation for Infantile Paralysis, 1940.

†Neoplastic Diseases. A Treatise on Tumors. By James Ewing, A.M., M.D., Sc.D., LL.D., Professor of Oncology at Cornell University Medical College, New York City; Consulting Pathologist, Memorial Hospital. Cloth, ed. 4, 1,160 pages, revised and enlarged, with 581 illustrations. W. B. Saunders Co., Philadelphia and London, 1940.

‡An Introduction to Biochemistry. By William Robert Fearon, M.A., Sc.D., M.B., F.I.C., Fellow of Trinity College, Dublin, Member of the Royal Irish Academy. Cloth, ed. 2, 475 pages, The C. V. Mosby Co., St. Louis, Mo., 1940.

After a rapid review of pertinent aspects of inorganic chemistry, the author turns to a more detailed study of organic compounds. Analytical methods are afforded their place in the discussion of each substance.

Written in an easy style, the book is graced by classical quotations some of which are especially apt. Over the index for instance, one sees "how index-learning turns no student pale, yet holds the eel of science by the tail" (Pope). This book may be heartily recommended.

Edible Wild Plants*

IF I AM ever lost in the forest and must subsist on what foods may be had from the wild plants about me, I shall want to have this book with me. It will tell me how to recognize and identify those wild plants which are edible, thus avoiding those which may be highly poisonous.

I will find a large variety for my menu, which will include 69 species of edible fruits and berries, 26 of salad plants and potherbs, 19 of roots and tubers, 15 of nuts, 13 of beverages and flavoring plants, 10 of seeds and seed pods, all minutely described, and a large number of others more briefly listed. Not only that, but if I am so fortunate as to have the book with me, I will find directions how to prepare or cook these plants.

There was a time when our pioneering ancestors realized the vital importance of this information. Now, when we are all eating cultivated foods, the knowledge has been all but lost.

Dr. Medsger has spent thirty years assembling the information recorded in his volume. It is profusely illustrated. It should be equally valuable to botanists and to those whose primary interest is in foods.

Compendium of Regional Diagnosis in Lesions of the Brain and Spinal Cord†

ACH year sees new books in medicine, each representing a new approach or interpretation. When the author's novel concept is good and is well grounded, the volume achieves popularity. With the lapse of time, others working in the same field may have better, more modern or more practical ideas concerning the presentation of the subject under discussion. When this occurs, the earlier texts, no matter how excellent they may have been, may pass into eclipse.

Some of them so adequately fulfill the need for which they were prepared that they outlive volumes which are submitted as more modern and may have been designed to supplant them. Such a work is Bing and Haymaker's regional diagnosis. After thirty years it still remains the preferred *vade mecum* for students for neuro-anatomy and neurologic localization.

There are several reasons. The authors' original manner of presentation filled a need. This having been achieved, there has been no attempt to pad successive editions, or to cover additional and less pertinent aspects. The book is still a small volume. The illustrations, many of which are in color, are quite completely descriptive and are sufficiently numerous to cover all phases of the subject. The general make-up of the book facilitates easy reading.

This volume is equally valuable to the freshman student learning neuro-anatomy, the neurologist and neurosurgeon, and the internist and general practitioner who require a ready reference work dealing primarily with the localization of lesions of the central nervous system.

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*Edible Wild Plants. By Oliver Perry Medsger, Professor Emeritus of Nature Education, Pennsylvania State College. With an Introduction by Ernest Thompson Seton. Cloth, 323 pages, illustrated with 80 pen and ink drawings and 19 photographs, $3.50. The Macmillan Co., New York, N. Y., 1939.

†Compendium of Regional Diagnosis in Lesions of the Brain and Spinal Cord. A Concise Introduction to the Principles of Localization of Diseases and Injuries of the Nervous System. By Robert Bing, Professor of Neurology, University of Basel, Switzerland. Translated and Edited by Webb Haymaker, Assistant Clinical Professor of Neurology and Lecturer in Neuro-Anatomy, University of California. Cloth, ed. 11, 292 pages, with 125 illustrations, 27 in color, and 7 plates, The C. V. Mosby Co., St. Louis, Mo., 1940.